Università di Pisa

Tesi di Dottorato di Ricerca in

Fisiopatologia della Riproduzione Umana e Sessuologia

A.A. 2010-2011

Titolo:

NEUROBIOLOGICAL EFFECTS OF DHEA IN FEMALES WITH SPECIAL REFERENCE TO SEXUAL FUNCTION: FINDINGS FROM *IN-VIVO* AND HUMAN STUDIES

Candidato: Dr. Nicola Pluchino

Relatore: Prof. A.R. Genazzani

ABSTRACT

Dehydroepiandrosterone (DHEA) and its sulfate ester, DHEAS, together represent the most abundant steroid hormones in the human body. Nonetheless, their physiological significance, their mechanisms of action and their possible roles in human disease are not well understood. Highlighting the potential health significance of DHEA and DHEAS, concentrations of these hormones in humans typically decrease steadily with age, approaching a nadir at about the time many diseases of aging become markedly more prevalent. There is growing evidence in the literature that a low DHEAS level, negatively correlates with the domains of sexual function in pre and postmenopausal women to a greater extent than testosterone levels. Biological actions of DHEA(S) involve neuroprotection, neurite growth, neurogenesis and neuronal survival, apoptosis, catecholamine synthesis and secretion, as well as anti-oxidant, anti-inflammatory and anti-glucocorticoid effects. In addition, DHEA affects neurosteroidogenis and endorphin synthesis/release. We demonstrated in a model of ovariectomized rats that DHEA therapy increases proceptive behaviors, already after 1 week of treatment, affecting central function of sexual drive. In women, the analyses of clinical outcomes are far from being conclusive and many issues should still be addressed. Although DHEA preparations have been available in the market since the 1990s, there are very few definitive reports on the biological functions of this steroid, and it is still the case that its regulation is unclear and its mechanisms of action largely yet to be established. We demonstrate that one year DHEA administration at the dose of 10 mg provided a significant improvement in comparison with vitamin D in sexual function and in frequency of sexual intercourse in early postmenopausal women. Among symptomatic women, the spectrum of symptoms responding to DHEA requires further investigation, to define the type of sexual symptoms (e.g. decreased sexual function or hypoactive sexual desire disorder) and the degree of mood/cognitive symptoms that could be responsive to hormonal treatment. In this regard, our findings are promising, although they need further exploration with a larger and more representative sample size.

INDEX

1. INTRODUCTION

The growth, differentiation, normal physiology and aging of the CNS are all now recognized to be influenced by gonadal steroid hormones. Steroids arriving from the gonads via the circulation modulate the responses of the brain, affecting not only sex behavior and sexually differentiated stereotypical behavioral responses, but also the ability of the brain to process, store and retrieve sensory information.

Dehydroepiandrosterone (DHEA) and its sulfate ester, DHEAS, together represent the most abundant steroid hormones in the human body. Nonetheless, their physiological significance, their mechanisms of action and their possible roles in human disease are not well understood. Highlighting the potential health significance of DHEA and DHEAS, concentrations of these hormones in humans typically decrease steadily with age, approaching a nadir at about the time many diseases of aging become markedly more prevalent. Observations such as these, coupled with basic and preclinical demonstrations of DHEA's biological effects, fostered hope that restoring DHEA to youthful levels might, conservatively, increase well-being and, optimistically, extend life, protect the brain, and retard the ravages of aging. Almost from the time of their initial discovery and synthesis, DHEA and DHEAS were evaluated in the treatment of neuropsychiatric disorders, with published reports appearing as early as 1952. Large-scale enthusiasm for DHEA as a potential neuropsychiatric therapy languished until the late 1980s through the mid-1990s, when an expanding body of preclinical data plus the first adequately controlled clinical trial renewed hopes for therapeutic potential.

Sexuality is determined by both intrinsic and extrinsic variables; and midlife is a multifaceted stage of woman's developments, characterized by important transitions. The difference between aging and sex in men and women is that women, experiment a menopause transition in which the hormone changes will occur in a relative short period; and in men the hormonal changes occur gradually during a more longer period. It is important to determine whether changes in women's sexual functioning during midlife are due to aging or to menopause. Recent research suggesting that a high proportion of men and women remain sexually active in later life refutes the beliefs of most of the cultures, that women become sexually retired or that sexual interest declines with age.

Though a large attention has been given to the study of postmenopause and to the options in hormone replacement treatment (i.e. estrogens and progestins), relative attention and awareness has been focused on the activity of endogenous or exogenous androgens in women. In fact the middle age of women life is characterized by the coexistence of menopause and adrenopause that

sometimes both participate to create the androgen-deficiency syndrome. Thus, much more interested have been launched to the study of androgen role in the modulation of brain function in term of sexuality, mood, cognition and neuroaging process. In these terms, the field of inquiry into the neurobiological actions of DHEA and DHEAS is rapidly growing.

The present thesis is to review briefly basic and preclinical studies of DHEA(S) biological actions in the brain and their supposed mechanisms of action, (2) to evaluate DHEAS specific effects on sexual function *in vivo*, and (3) the therapeutic potential of DHEA(S) in postmenopausal women using on measure of quality of life and sexual function.

2. DHEA(S) SYNTHESIS AND METABOLISM

Dehydroepiandrosterone, 5-androsten-3 beta-ol-17-one, is a 19 carbon steroid that is synthesized from cholesterol by two steroid metabolizing enzymes (1). The first, rate-limiting, and hormonally regulated step in the synthesis of all steroid hormones is the conversion of cholesterol into pregnenolone by the mitochondrial enzyme cholesterol side chain cleavage P450scc. Pregnenolone is converted into DHEA by the enzyme cytochrome P450c17; this single enzyme catalyzes both the 17α-hydroxylation reaction converting pregnenolone to 17-OH pregnenolone and the 17,20-lyase reaction converting 17-OH pregnenolone to DHEA (2). The sulfation of DHEA into its more stable sulfate ester DHEAS is catalyzed by the enzyme hydroxysteroid sulfotransferase (HST, SULT2A1), commonly known as DHEA sulfotransferase. DHEAS can be converted back into DHEA by steroid sulfatase (STS).

People with 17α-hydroxylase deficiency are characterized by sexual infantilism in phenotypic females (due to lack of sex steroid precursors), 46,XY disorder of sexual development (lack of masculinization – female infantile external genitalia, no uterus), hypertension, and hyperkalemia (3). P450c17 is encoded by a single gene (cyp17) and mutations can cause either 17α-hydroxylase deficiency or 17,20-lyase deficiency or both (3). In addition to its expression in human adrenals and gonads, P450c17 is also expressed in the brain (4), where it may synthesize DHEA from pregnenolone. There are no reported neurological problems in people with P450c17 gene mutations, perhaps because they obtain sufficient quantities of 17-hydroxylated steroids from their mothers during prenatal development. Adults with P450c17 gene mutations are not well studied and may be an interesting group to examine with regard to neuropsychiatric illness, although this could be complicated with the possible psychological effects of sexual infantilism. Mouse studies knocking out this gene were uninformative, as the P450c17−/− mice died by embryonic day 7 before gastrulation, and the cause of this early lethality is unknown (5).

Adrenal secretion of DHEA and DHEA-S increases during adrenarche in children at the age of 6–8 years. Maximal values of circulating DHEA-S are reached between the ages of 20 and 30 years. Thereafter, serum DHEA and DHEA-S levels decrease markedly (6-7). In fact, at 70 years of age, serum DHEA-S levels are decreased to approximately 20% of their peak values, while they can decrease by 95% by the age of 85–90 years.

The marked reduction in the formation of DHEA-S by the adrenals during aging (6-7) results in a dramatic fall in the formation of androgens and estrogens in peripheral target tissues.

Transformation of the adrenal precursor steroids DHEA-S and DHEA into androgens and/or estrogens in peripheral target tissues depends upon the level of expression of the various steroidogenic and metabolizing enzymes in each cell of these tissues. This sector of endocrinology that focuses on the intracellular hormone formation and action has been called intracrinology (8) (Fig.2). This situation of a high secretion rate of adrenal precursor sex steroids in men and women is thus completely different from all animal models used in the laboratory, namely rats, mice, guinea pigs and all others (except monkeys), where the secretion of sex steroids takes place exclusively in the gonads (7). One explanation for the delayed progress in the field of formation of sex steroids in peripheral target tissues or intracrinology is the fact that the adrenals of the animal models usually used do not secrete significant amounts of adrenal precursor sex steroids, thus focusing all attention on the testes and ovaries as the exclusive sources of androgens and estrogens. The term intracrinology was thus coined (8) to describe the synthesis of active steroids in peripheral target tissues where the action is exerted in the same cells where synthesis takes place without release of the active steroids in the extracellular space and general circulation (8).

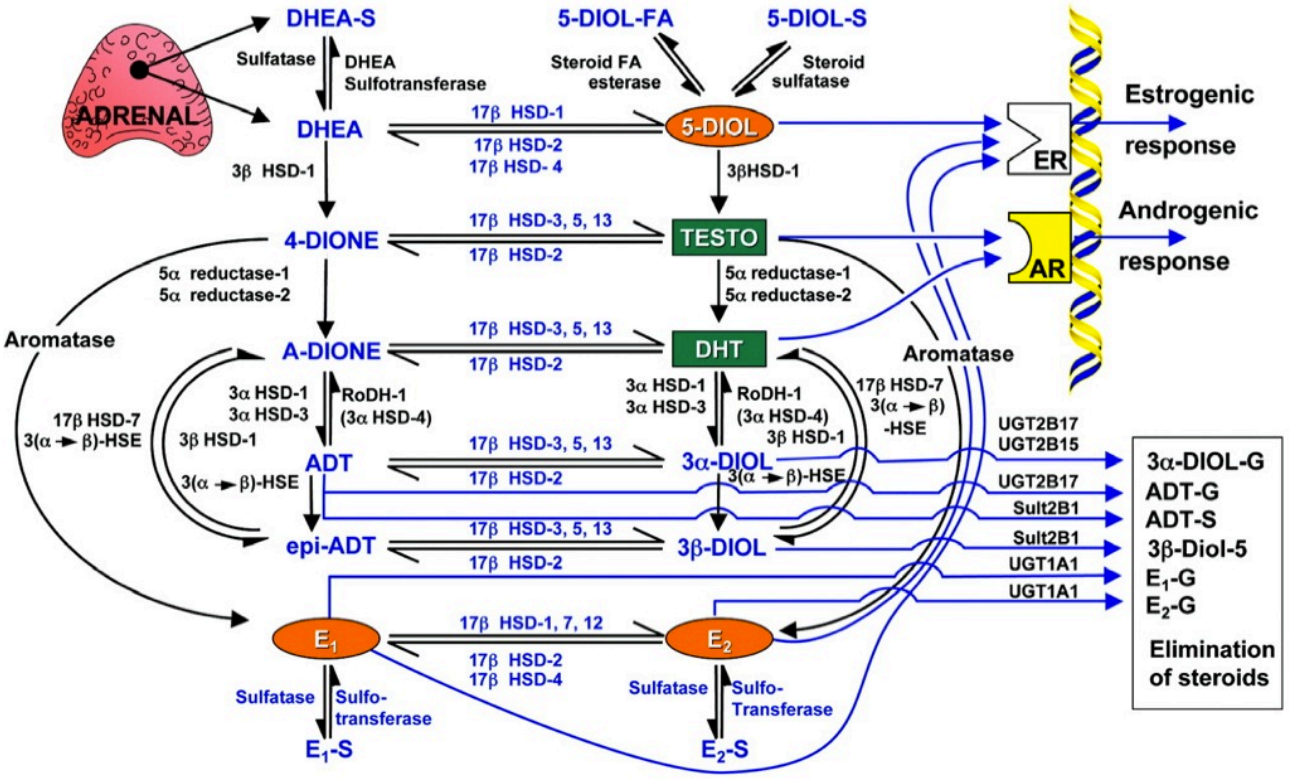


Fig1. Human steroidogenic and steroid-inactivating enzymes in peripheral intracrine tissues. 4-DIONE, androstenedione; A-DIONE, 5-alpha-androstane-3,17-dione; ADT, androsterone; epi-ADT, epiandrosterone; E_1, estrone; E_1-S, estrone sulfate; 5-DIOL-FA, androst-5-ene-3alpha,17beta-diol fatty acid; 5-DIOL-S, androst-5-ene-3alpha,17beta-diol sulphate; HSD, hydroxysteroid dehydrogenase; TESTO, testosterone; RoDH-1, Ro dehydrogenase 1; ER, estrogen receptor; AR, androgen receptor; UGT2B28, uridine glucuronosyl transferase 2B28; Sult2B1, sulfotransferase 2B1; UGT1A1, uridine glucuronosyl transferase 1A1.

The almost exclusive focus on the role of ovarian estrogens in women's reproductive physiology has removed attention from the dramatic 70% fall in circulating DHEA which already occurs between the ages of 20 to 30 and 40 to 50 years In fact, since DHEA is transformed to both androgens and estrogens in peripheral tissues, such a fall in serum DHEA and DHEA-S explains why women at menopause are not only lacking estrogens but are also likely to have been deprived of androgens for a few years, as illustrated by the 50–60% decrease in serum ADT-G (9) (Fig. 1).

In a recent study nine androgens and their precursors and metabolites were measured by gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry in serum samples from 377 healthy postmenopausal women aged 55–65 years and 47 normally cycling 30- to 35-year-old premenopausal women. A decrease of 60% was then observed in the sum of ADT-G and 3α-diol-G while serum DHEA was decreased by 54% in postmenopausal compared with premenopausal women (8). Serum testosterone, on the other hand, did not decrease significantly from 0.18 ± 0.07 in premenopausal to 0.14 ± 0.07 ng/ml in postmenopausal women.

Since the serum levels of ADT-G and 3α-diol-G in women are 70% of those found in men of the same age while serum testosterone in women compared with men is only about 3% (0.15 ng/ml in women versus 4.5 ng/ml in men), it is clear that serum testosterone is not a valid marker of androgenicity in women. This situation is somewhat analogous to the situation in castrated men where castration causes a 90–95% reduction in the concentration of serum testosterone while the intra-prostatic concentration of DHT as well as of serum ADT-G and 3α-diol-G are only reduced by 50–70% (10).

Completion of the identification and characterization of all the human UDP-glucuronosyl transferases has made possible the use of the glucuronide derivatives of androgens as markers of androgenic activity. In fact, UGT2B7, UGT2B15 and UGT2B17 are the three enzymes responsible for the glucuronidation of all androgens and their metabolites in the human (11). The relatively simple inactivation mechanisms of androgens permits measurement of the sum of the metabolites of all androgens in the circulation, thus offering a precise assessment of the total androgenic activity in both women and men.

While the only means of determining androgenic activity in specific tissues is the direct measurement of the intra-tissular concentration of the active androgens, such measurements are not possible in the human except under exceptional circumstances such as in samples of cancer tissue obtained at surgery (7-9) However, while not permitting the assessment of androgenic activity in specific tissues, measurement by validated mass spectrometry techniques of the glucuronide

derivatives of ADT and 3α-diol permits an accurate assessment of total androgenic activity in the whole organism. In fact, since inactivation of the active androgens into ADT and 3α-diol and their subsequent glucuronidation into ADT-G and 3α-diol-G is the obligatory route of elimination of androgens (11-12) this approach appears to be the best means of evaluating total androgenic activity in individual subjects and patients. The clinician can then reliably correlate these values of androgenic activity with the other clinical findings.

As mentioned above, the level of transformation of the adrenal precursor steroid DHEA into androgens and/or estrogens in peripheral target tissues depends upon the level of expression of the various steroidogenic enzymes in each cell of each of these tissues (8). This situation of a high secretion rate of adrenal precursor sex steroids by the adrenals in men and women is thus completely different from all animal models used in the laboratory, namely rats, mice, guinea pigs and all others (except monkeys), where the secretion of sex steroids takes place exclusively in the gonads and the adrenals do not secrete significant amounts of DHEA (14).

The classical concept of androgen and estrogen secretion in women assumed that all sex steroids had to be transported by the general circulation following secretion by the ovaries before reaching the target tissues. According to this classical concept, it was erroneously believed that the active steroids could be measured directly in the circulation, thus providing a potentially valid measure of the general exposure of the whole body to sex steroids. In fact, this concept is valid only for animal species lower than primates but it does not apply to the human, especially in postmenopausal women where all estrogens and almost all androgens are made locally from DHEA in the peripheral tissues, which possess the enzymes required to synthesize active sex steroids. Such a local biosynthesis and action of androgens in target tissues eliminates the exposure of other tissues to androgens and thus minimizes the risks of undesirable masculinizing or other androgen-related side-effects. The same applies to estrogens, although we feel that a reliable parameter of total estrogen secretion (comparable with the glucuronides identified for androgens) has yet to be determined.

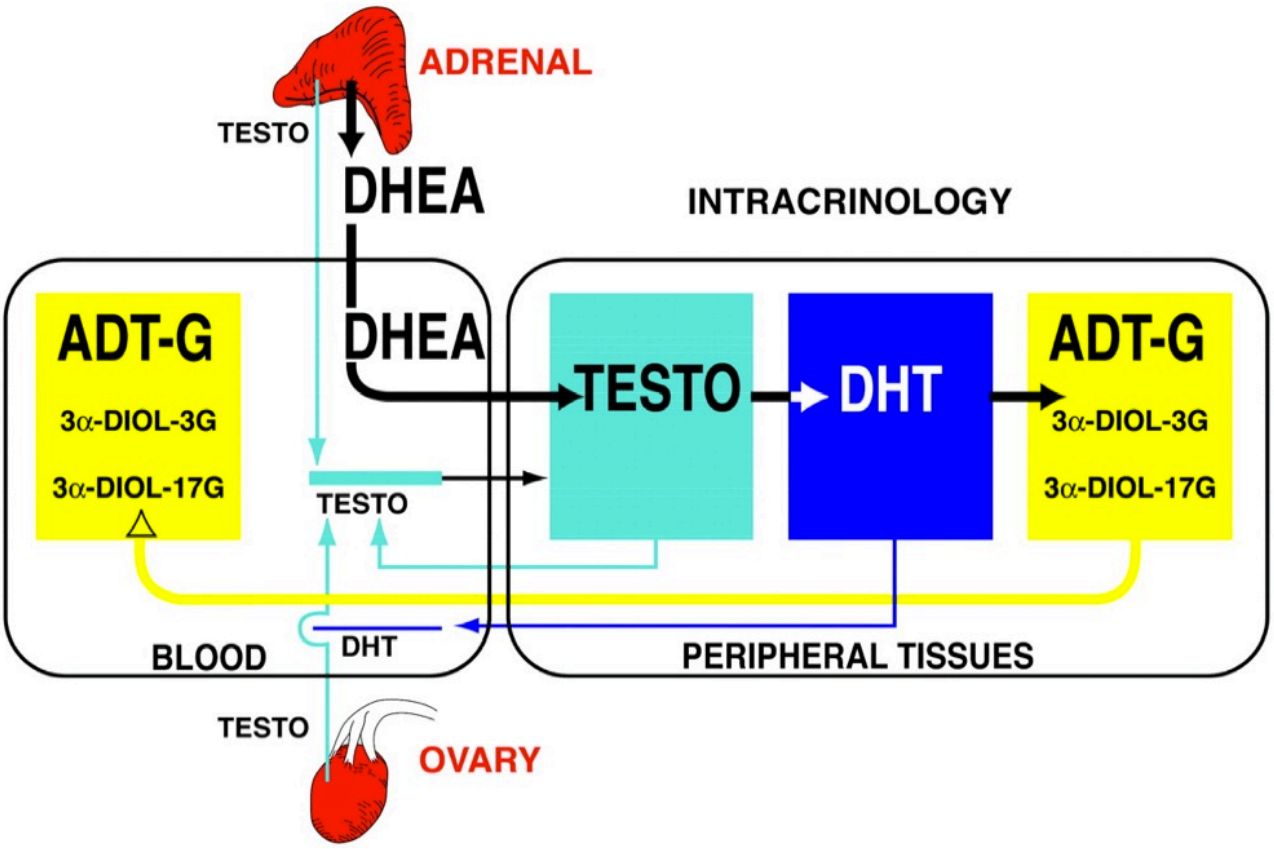


Fig. 2 Schematic representation of the very important contribution of the precursor DHEA of adrenal origin to total androgenic activity in postmenopausal women with a parallel minor contribution of testosterone (TESTO) of ovarian and adrenal origins. By intracrine mechanisms, DHEA is transformed into testosterone and DHT in peripheral tissues and then into the inactive metabolites ADT and 3α-diol before transformation into the water soluble glucuronide derivatives ADT-G, 3α-diol-3 G and 3α-diol-17 G by the UGTs 2B7, 2B15 and 2B17. These water-soluble metabolites are then released into the general circulation where they can be measured. A very small proportion of the testosterone and DHT made intracellularly by the steroidogenic enzymes of the intracrine pathway diffuse into the circulation. The height of the colored boxes is proportional to the concentration of each steroid.

2.1 Relative DHEA(S) Concentrations in Brain vs. Plasma vs. CSF in Humans

Higher concentrations of DHEA are found in the brain compared to plasma. In a study of ten postmortem human brains, DHEA concentrations were 29.4 nmol/kg in prefrontal lobe, 16.3 nmol/kg in parietal lobe, 13.1 nmol/kg in temporal cortex, 16.9 nmol/kg in cerebellum, and 18.7 nmol/kg in corpus callosum (15). These data were derived from nine women and one main (76–93 years old), and it is worth noting that large individual differences in DHEA brain concentrations were observed, with prefrontal lobe DHEA concentrations ranging from 9.8 to 470 nmol/kg (16). Mean DHEA concentrations were 1.83 nM in plasma of living human subjects of similar ages, which results in a brain-to-plasma ratio of ~6.5 (16). Although human brain concentrations of DHEA are higher than plasma concentrations, cerebrospinal fluid (CSF) concentrations of DHEA are lower than plasma concentrations. DHEA concentrations in CSF were ~5% of those found in the plasma of humans (16).

The validity of reported measurements of DHEAS and pregnenolone sulfate in the brain has recently been questioned (17,18). Many studies have relied on identification of parent compounds after separation of steroid sulfates from free steroids by organic:aqueous solvent extraction followed by a chemical reaction (solvolysis) to remove the sulfate. Analyses of sulfated steroids after extraction and solvolysis have found high concentrations of DHEAS and pregnenolone sulfate in rodent and human brains (19-20). Recent studies that measure intact sulfated compounds without deconjugation However, high DHEAS concentrations were found in two samples of human brain tissue using the new sample preparation method described above and gas chromatography-mass spectrometry (GC-MS) analysis (21). Hence, humans may indeed have high concentrations of brain DHEAS and older studies may turn out to be correct once verified using these newer analytic protocols (22,23). Studies relying solely on organic:aqueous extractions and solvolysis to measure DHEAS remain questionable and need to be reassessed.

2.2. Species Differences - Humans vs. Rodents

Humans and rodents (rats and mice) differ in the pathways through which sex steroids are synthesized. Whereas the Δ4 pathway predominates with rodents, the 17,20-lyase activity of the human P450c17 enzyme strongly prefers the Δ5 pathway (24). Subsequent conversion of DHEA into androstenedione by 3β-hydroxysteroid dehydrogenase (3βHSD) is the only pathway by which humans produce androstenedione (25). In rodents, conversion of cholesterol to androstenedione can occur through two pathways – the Δ5 pathway described above and the Δ4 pathway which involves the conversion of pregnenolone into progesterone (by 3βHSD) and progesterone conversion into androstenedione through the 17-OH-progesterone intermediary. Thus, humans make DHEA (Δ5 pathway) prior to downstream conversion into androstenedione and further metabolism into other sex steroids, whereas rodents go through the Δ4 pathway (predominantly) or Δ5 pathway. The species difference in predominant steroid pathways may partly explain species differences in peripheral circulating concentrations. Whereas DHEAS is the most abundant circulating steroid hormone in the human body (26), rats and mice (the species typically studied) have low circulating concentrations of DHEA(S) in the periphery (27). Unlike humans who secrete DHEA(S) from their adrenal glands and gonads, rats and mice can only synthesize and secrete DHEA(S) from their gonads, as their adrenal glands lack P450c17 (28).

Like humans, rats and mice have higher concentrations of DHEA in the brain compared to the plasma (4). For example, Sprague-Dawley rats had mean DHEA concentrations of 0.08 ng/ml (0.28 nM) in plasma, while brain concentrations of DHEA were 0.42 ng/g (1.46 nmol/kg) in anterior brain and 0.12 ng/g (0.42 nmol/kg) in posterior brain (4). These data are consistent with the hypothesis that in rodents, brain DHEA is derived mainly if not solely from local synthesis and not from peripheral synthesis. In human beings, brain DHEA may be derived from both local synthesis and peripheral synthesis. Thus, since DHEA is found in appreciable concentrations in brains of both human beings and rodents, rodents may indeed be a good model for studying the function of DHEA in the brain, but may not be an appropriate model for studying peripheral effects of these steroids.

3. DHEA(S) AS A NEUROSTEROID.

Important actions in the central nervous system (CNS) were initially inferred from observations that DHEA and DHEAS were synthesized de novo in brain, as brain concentrations were higher than plasma concentrations and brain concentrations remained high after adrenalectomy and gonadectomy of rats (4). Indeed, they have been termed "neurosteroids" for this reason (29-30). DHEA and DHEAS were among the first neurosteroids identified in rat brains. Cytochrome P450c17 was found in a subset of neurons of embryonic rodent brains (29). P450c17 expression was mainly neuronal; its expression was found as early as embryonic day 9.5, and persisted in the CNS during embryonic development. In one study, P450c17 was not detected in the CNS in adult rats and mice by immunocytochemistry, raising the possibility that this enzyme, and its neurosteroid products, function mainly during development (31). However, another study found P450c17 in adult male rat hippocampus by immunohistochemical staining (31). In the hippocampus, P450c17 was localized to pyramidal neurons in the CA1-CA3 region and to granule cells of the dentate gyrus. In these cells, P450c17 was localized in pre- and post-synaptic locations and in the endoplasmic reticulum by immunoelectron microscope analysis. While P450c17 protein was readily detected in the brain, the abundance of P450c17 mRNA transcripts in the embryonic mouse brain (31) or hippocampus of adult male rats was low, and was approximated to be 1/200th of the expression in the testis.

DHEA can be synthesized in vivo in rat and frog brains. Rat brains were capable of converting pregnenolone into DHEA and this may be activity-dependent (32). Basal P450c17 steroidogenic enzyme activity was low in the hippocampus, but could be enhanced by exposing neurons to N-methyl-D-aspartate (NMDA) (32). Similar findings have been reported for NMDA stimulation of pregnenolone synthesis from cholesterol in the hippocampus (33), suggesting that both P450scc and P450c17 are regulated by neurotransmitters. Frog brains also were found to synthesize DHEA from pregnenolone, and this enzymatic activity was reduced in a concentration-dependent manner by ketoconazole, an inhibitor of P450c17 (34). P450c17 enzymatic activity and protein expression were co-localized, further indicating that the enzymatic activity was due to P450c17.

P450c17 expression has also been found in adult rat spinal cord. Immunohistochemical studies localized P450c17 in both neurons and glial cells in the spinal cord. Slices of spinal cord tissue containing P450c17 protein converted (3H)pregnenolone into (3H)DHEA, and this conversion was reduced by ketoconazole. Thus, the spinal cord is one region in the CNS of rodents that expresses P450c17 and can synthesize DHEA endogenously from a precursor (35).

DHEAS may be synthesized in the brain from DHEA (36). Sulfation of DHEA has been observed in the brains of rhesus monkeys in vivo and in human fetal brain slices in vitro (36). Conversion of (3H)DHEA into (3H)DHEAS was also found in incubations of brain homogenates from pons, hypothalamus, olfactory bulb, cortex, and striatum/hippocampus of fetal and adult Sprague-Dawley rats and from thalamus, frontal cortex, basal ganglia, olfactory bulb, hippocampus, brainstem, midbrain, occipital cortex and cerebellum of adult Wistar rats . In addition to mammals, DHEAS synthesis from DHEA has been observed in brain homogenates from hypothalamus and telencephalon but not rhombencephalon of adult European green frogs.

Hydroxysteroid sulfotransferase (HST) or SULT2A1, also commonly referred to as DHEA sulfotransferase, is an enzyme that sulfonates DHEA (in addition to pregnenolone) (37). Western blotting and immunohistochemistry (with an antibody directed against partially purified rat liver HST) showed protein expression of an HST in adult Wistar rat brain (3). However, the characterization of this HST was not fully addressed, and hence its identity was uncertain. Other studies using different antibodies to purified or well-characterized proteins have confirmed the finding of HST in the brains of rats and frog. SULT2A1 mRNA expression has been shown in rat brains, thereby definitively demonstrating the presence of SULT2A1 in the brain. Future research on the activity and localization of newly discovered sulfotransferases, such as SULT2B and SULT4, may further our understanding of DHEA sulfonation in the brains of humans, rats and mice in the future (37).

It is unlikely that brain DHEAS comes from the periphery because sulfated steroids are hydrophilic and do not readily cross the blood-brain barrier, as evidenced by low recovery (0.03%) of radioactively labeled DHEAS in the brains of Sprague-Dawley rats following intracardiac injection (154). Although, one study has found increased pregnenolone sulfate in the brains of Sprague-Dawley rats after i.v. injection via the tail vein (325). What little steroid sulfates do enter the brain may occur through organic anion transporting peptides (OATP), which may work to transport DHEAS in both directions (13). However, steroid sulfates may egress from the brain more readily than they enter. The efflux clearance of (3H)DHEAS across the blood-brain barrier was determined to be tenfold greater than its influx (118 (µl/min-g efflux vs. 11.4 µl/min-g influx) (13). Hence, DHEAS is predominately transported out of the brain across the blood-brain barrier, further suggesting that DHEAS found in the brain is most likely due to local synthesis.

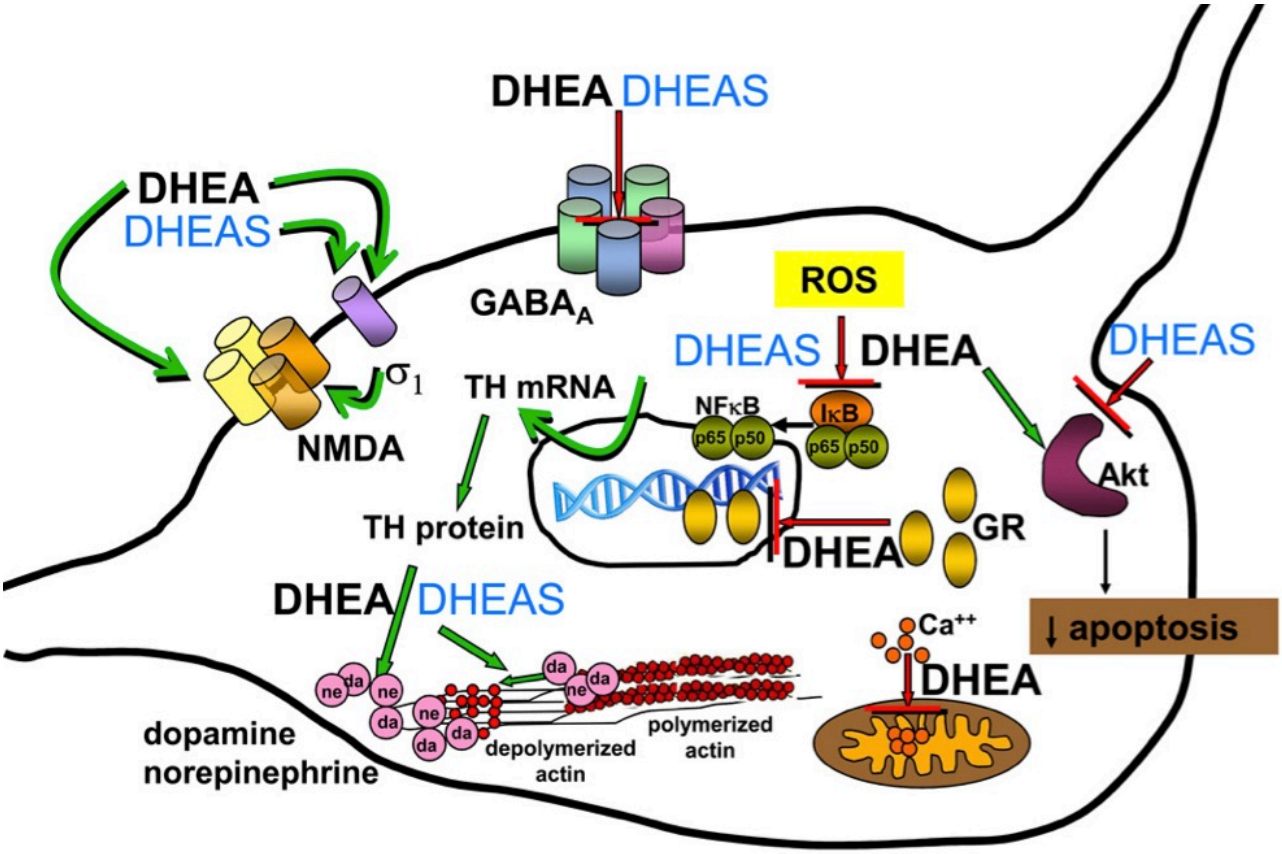
4. DHEA(S) MECHANISMS OF ACTION

Steroid hormones affect gene transcription by binding to specific cytoplasmic receptors, and then translocating into the nucleus, or binding to receptors that are resident in the nucleus, where they bind to steroid responsive elements on DNA. To date, no nuclear steroid receptor with high affinity for either DHEA or DHEAS has been found (38-40). The mechanisms by which DHEA(S) operate are not fully understood (38). DHEA(S) may mediate some of its actions through conversion into more potent sex steroids and activation of androgen or estrogen receptors in tissue (i.e. skin, liver, brain) (41). In addition to DHEA(S) having effects through its sex steroid metabolites (i.e. estradiol and testosterone), DHEA(S) may also have effects through its more immediate metabolites, such as 7α-hydroxy-DHEA (42). Although no unique DHEA or DHEAS nuclear steroid receptor has been found, DHEA and DHEAS have been found to affect receptors and to show affinity for some binding sites (38).

In the brain, DHEA(S) modulates actions of the γ-aminobutyric acid type A (GABAA) receptor, the NMDA receptor, and the sigma subtype 1 (σ1) receptor (43-48) among others (49-51). DHEA and DHEAS generally act as noncompetitive antagonists at the GABAA receptor, with DHEAS having more potent antagonistic effects than DHEA (52-53). DHEA(S) generally acts as a positive allosteric modulator of the NMDA receptor, although the binding of DHEA(S) with an interaction site on the NMDA receptor is not well documented (43,46). DHEA(S) can potentiate NMDA receptor function through its actions as a σ1 receptor agonist. However, in non-hippocampal brain regions DHEA(S) may inhibit glutamate neurotransmission through σ receptors, since σ receptor agonists were shown to reduce NMDA-induced dopamine release in the striatum (54). In an electrophysiological study with Sprague-Dawley rats, intravenous (i.v.) administration of DHEA (100–500 μg/kg) potentiated the NMDA neuronal response of CA3 rat hippocampus pyramidal neurons in a dose-dependent manner (32). The addition of σ receptor antagonist haloperidol or σ1 receptor antagonist N-dipropyl-2-(4-methoxy-3-(2-phenylethoxy)phenyl)-ethylamine monohydrochloride (NE-100), but not saline or spiperone (which has low affinity for σ receptors), inhibited the potentiating effect of DHEA, suggesting that DHEA can modulate the NMDA response through σ1 receptors (55). DHEAS potentiated the NMDA evoked release of (3H)norepinephrine from preloaded hippocampal slices, while the addition of σ receptor antagonists haloperidol or 1-(2-(3,4-dichlorophenyl)-ethyl)-4-methylpiperazine (BD1063) blocked the potentiating effect of DHEAS (56). Thus, DHEA(S) can modulate NMDA neurons and receptor activity by acting at the σ1 receptor (that is coupled to Gi/o proteins) in both in vivo and in vitro studies (55,56).

DHEAS, but not DHEA, augments cholinergic function in several animal models (57). Intraperitoneal (i.p.) administration of DHEAS (25–250 μmol/kg) increased acetylcholine (ACh) release from hippocampal neurons in rats (58). This effect has behavioral relevance in vivo, since DHEAS prevented (in a dose-dependent manner) the memory impairment induced by the ACh receptor antagonist scopolamine in mice (59). The σ1 receptor antagonist NE-100 blocked the ameliorating effects of DHEAS in this model, suggesting that the modulation of the cholinergic system by DHEAS involves interaction with σ1 receptors (59). Long term administration (15 days) of the STS inhibitor p-O-(sulfamoyl)-N-tetradecanoyl tyramine (DU-14) (which inhibits the conversion of DHEAS to DHEA) to rats increased plasma DHEAS concentrations, decreased DHEA concentrations, increased hippocampal ACh release, and blocked scopolamine-induced amnesia (60).

Additional intracellular sites where DHEA may act have also been described. DHEA may interact directly with certain cytoskeleton components or novel membrane receptors. DHEA was found to bind to microtubule-associated protein (MAP) 2C with strong affinity (61). MAP2C, which is expressed at early development stages, was found in adult retina and olfactory bulb, which are tissues in which neurogenesis persists in the adult (61). Intriguing leads are emerging for possible DHEA receptor sites in the periphery that may also exist in the central nervous system. A DHEA receptor was found on endothelial cell plasma membranes and it was coupled to endothelial nitric-oxide synthase (eNOS) activity through Gi/o proteins Gαi2 and Gαi3 (62). DHEA(S) may also have actions at other receptors, including the peroxisome proliferator-activated receptor α (PPARα), pregnane X receptor, constitutive androstanol receptor, and estrogen receptor β (63-65).



Mechanisms of action of DHEA and DHEAS in neurons. This cartoon summarizes many of the actions of DHEA and DHEAS described in detail in the text. DHEA and DHEAS have inhibitory effects (red blocking arrow) at the $GABA_A$ receptor (section 6 and 7.1). DHEA and DHEAS act as agonists (green arrow) at the σ_1 receptor (section 6 and 7.1), which subsequently may activate the NMDA receptor. DHEA inhibits Ca^{2+} influx (red blocking arrow) into the mitochondria (section 7.1). DHEA influences embryonic neurite growth through stimulation (green arrow) of the NMDA receptor (section 7.2). DHEA increases (green arrow) kinase activity of Akt and decreases apoptosis, while DHEAS decreases (red blocking arrow) Akt and increases apoptosis (section 7.4). DHEAS increases (green arrows) TH mRNA and TH protein abundance (section 7.5) leading to increased catecholamine synthesis. DHEA and DHEAS stimulate (green arrows) actin depolymerization and submembrane actin filament disassembly and (green arrows), increasing secretion of catecholamines ("da" and "ne") from secretory vesicles (section 7.5). DHEA and DHEAS inhibit (red blocking arrow) reactive oxygen species (ROS) activation of transcription mediated by NF-κB (section 7.6 and 7.7). DHEA inhibits (red blocking arrow) nuclear translocation of the glucocorticoid receptor (GR) (section 7.8). Mechanisms of action not pictured in this graph are: alterations of brain derived neurotrophic factor (BDNF) synthesis, inhibition of stress-activated protein kinase 3 (SAPK3) translocation, and inhibition of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSDl) activity. Abbreviations: σ_1, sigma 1 receptor; Akt, serine-threonine protein kinase Akt; Ca^{2+}, calcium; da, dopamine; $GABA_A$, γ-aminobutyric acid type A receptor; GR, glucocorticoid receptor; ne, norepinephrine; NF-κB, nuclear factor kappa B; NMDA, *N*-methyl-D-aspartate receptor; ROS, reactive oxygen species; TH, tyrosine hydroxylase.

5. NEUROBIOGICAL ACTION OF DHEA WITH POTENTIAL RELEVANCE TO SEXUAL FUNCTION: EXPERIMENTAL FINDINGS

Neurobiogical action of DHEA involve directy DHEAS, DHEA and its more immediate metabolites (e.g., 7α-hydroxy-DHEA) in the brain but they are also due to conversion of DHEA(S) into sex steroids (estradiol, testosterone, DHT, 3alpha-DIOL, 3Beta-DIOL). Neurobiological actions of estradiol and testosterone are well established (66-69), less characterized are the function attributable directly to DHEA and DHEAS. These major biological actions of DHEA(S) involve neuroprotection, neurite growth, neurogenesis and neuronal survival, apoptosis, catecholamine synthesis and secretion, as well as anti-oxidant, anti-inflammatory and anti-glucocorticoid effects. In addition, DHEA affects neurosteroidogenis and endorphin synthesis/release

5.1 DHEA and catecholamine synthesis/secretion

DHEA(S) influence catecholamine synthesis and secretion (70). DHEAS was protective against the neurotoxin MPP+ (which inhibits catecholamine synthesis and triggers cell death) in rat cerebellar granule cell cultures. In an in vivo study of long-term DHEA treatment, obese and lean female Zucker rats were fed chow containing 0.6% DHEA for 28 days (71). Lean rats had higher norepinephrine (NE) in the lateral hypothalamus but lower NE in the paraventricular nucleus (PVN) of the hypothalamus compared to lean rats fed the control diet (71). There were no differences among obese Zucker rats in NE, serotonin (5HT), and serotonin metabolite 5-hydroxyindole-3-acetic acid (5-HIAA) in the hypothalamus (71). In another study, obese female Zucker rats treated with a large, acute i.p. injection of DHEA (200 mg/kg) had increased concentrations of dopamine (DA), 5HT, and 5-HIAA, and decreased concentrations of NE and epinephrine (EPI) in the paraventricular nucleus compared to controls that received oil vehicle (72). DHEA treatment had no effect on neurotransmitter concentrations in the lateral hypothalamus or ventromedial hypothalamus (72). These in vivo studies suggest that the duration and/or dose of DHEA treatment may be important and that DHEA may have different effects on different parts of the hypothalamus. In vitro, DHEAS (10−8 M and 10−6 M) has been found to stimulate dopamine release from rat hypothalamic cells in primary cultures (73).

DHEA(S) affects proliferation of catecholamine-producing adrenomedullary chromaffin cells. Although DHEA and DHEAS do not induce proliferation by themselves, they may modulate proliferation induced by growth factors and do so in an age-dependent manner. In bovine chromaffin cells from young animals, DHEA decreased cell proliferation induced by insulin-like growth factor-II (IGF-II), but had no effect on proliferation induced by basic fibroblast growth

factor (bFGF) (74). In another study, DHEA decreased cell proliferation induced by leukemia inhibiting factor (LIF) in bovine chromaffin cells from young animals (75). In bovine chromaffin cells from adult animals, DHEA decreased cell proliferation induced by epidermal growth factor (EGF) (75). DHEAS had no effect on LIF-induced proliferation of cells from young animals, but high micromolar concentrations of DHEAS enhanced EGF-induced proliferation of cells from adults (75). The effects of DHEA and DHEAS were not due to downstream metabolism into sex steroids since neither the estrogen receptor antagonist ICI 182,780 nor the androgen receptor antagonist flutamide affected chromaffin cell proliferation (75). Thus, local production of DHEA and DHEAS in the adrenal cortex can influence proliferation of chromaffin cells, and may have similar effects on catecholamine-producing neurons in the brain.

DHEA and DHEAS stimulate secretion of catecholamines from rat pheochromocytoma PC12 cells and are involved in inhibition of neuronal proliferation and promotion of differentiation of adrenal medullary cells to a more neuroendocrine phenotype. Administration of nerve growth factor (NGF) induced PC12 cells to differentiate into a neuronal phenotype, while administration of DHEA alone had no effect (76). PC12 cells incubated with both NGF and DHEA had lower survival and less neurite outgrowth than cells incubated with NGF alone in serum-free medium (76). NGF dose-dependently induced phosphorylation of extracellular signal-regulated kinases (ERK)1/2, which distinguished proliferation from differentiation processes, and this ERK1/2 phosphorylation was inhibited by DHEA (76-77). Furthermore, DHEA stimulated dopamine release from NGF-treated cells, while neither NGF nor DHEA alone had an effect on dopamine release (77). Another study compared the stimulatory effects of DHEA and DHEAS on catecholamine synthesis. The effect of DHEA was faster than that of DHEAS; whereas the effect of DHEA peaked at 10 min, the effect of DHEAS peaked at 30 min (58). In addition to stimulating secretion, DHEAS (but not DHEA) also stimulated catecholamine production. DHEAS increased tyrosine hydroxylase (TH) protein abundance in PC12 cells after four hours of stimulation, and also increased TH mRNA abundance in PC12 cells after only two hours of stimulation (58). These data suggest that DHEA and DHEAS function differently, and that DHEAS may directly affect TH gene transcription. Experiments to determine if DHEA increases TH gene transcription directly have not yet been reported.

DHEA and DHEAS have non-transcriptional effects on catecholamine secretion. DHEA and DHEAS have been found to stimulate actin depolymerization and submembrane actin filament disassembly, a fast-response cellular system regulating trafficking of catecholamine vesicles (78). An actin meshwork inhibits catecholamine secretory vesicles from reaching exocytosis sites. By decreasing this actin meshwork, DHEA and DHEAS increase the ability of catecholamines to be

secreted from secretory vesicles. Addition of DHEA and DHEAS to PC12 cells induced actin depolymerization, as measured by the ratio of G-monomeric to total cellular actin, an established marker of actin cytoskeleton dynamics (78). When PC12 cells were exposed to phallacidin, an actin filament stabilizer, the stimulatory effect of DHEA and DHEAS on both dopamine and norepinephrine secretion was prevented (78). These studies show that DHEA and DHEAS exert a direct effect on PC-12 cells (a model of chromaffin cells), and thus, provide in vitro evidence of how the zona reticularis and the adrenal medulla may be interacting in vivo. These findings also raise the possibility that DHEA and DHEAS could increase catecholamine production and release in the brain.

5.2 DHEA allopregnanolone and beta-endorphin

Allopregnanolone is a 3-α, 5-α reduced metabolite of Progesterone (79), and its major sources are the gonads and adrenal cortex, and, to a lesser extent, the CNS (79). Allopregnanolone acts as an agonist of GABAA receptor, modulating stress, mood, and behavior. Gonadal steroids may modulate allopregnanolone levels, as suggested by several experimental studies on animal models. In fact, female rats show significantly higher hippocampal allopregnanolone concentrations on the morning and afternoon of proestrous than at diestrous or estrous, reaching the lowest levels at estrous. Moreover, ovariectomy determines an increased adrenal allopregnanolone content and a reduction in allopregnanolone levels in brain and serum; this may be due to an estrogen-mediated enzymatic induction in the synthesis of allopregnanolone (79-81).

β-endorphin is the most important and biologically active endogenous opioid peptide; it has behavioral, analgesic, thermoregulatory, and neuroendocrine properties. A decrease in central and peripheral β-EP levels in ovariectomized rats and in circulating β-EP levels in postmenopausal women has been shown (82)

Dehydroepiandrosterone administration induces an increase in β-EP content in anterior and neurointermediate pituitary and hippocampus in a dose-dependent manner. High-dose DHEA administration induces an increase in β-EP circulating levels to those observed in fertile animals and an even greater increase in the hypothalamus. Comparing these results with those assessed administering E2 in ovx rats, we can observe several similarities. In all the examined organs, the effect of DHEA on β-EP levels closely reflects that of E2 valerate, with the exception of the neurointermediate pituitary, where DHEA induces a lower increase in β-EP.

Dehydroepiandrosterone administration induces an increase in allopregnanolone content in the hypothalamus, anterior pituitary, serum, and hippocampus, where DHEA administration restores allopregnanolone levels to those observed in fertile rats. Similar data have been observed at the central level when administering E2 valerate in ovx rats. However, DHEA does not determine a decrease in allopregnanolone adrenal content, as observed with E2. Moreover, the serum allopregnanolone levels reached in response to DHEA therapy are lower than those obtained with E2 administration (83)

The mechanisms of action of DHEA are not clear. Part of the effects of DHEA depends on its conversion to estrogens and androgens and on the recruitment of their receptors. In fact, at the vascular level, a reduction of atherosclerotic lesions by DHEA in rabbits was reported, partially through conversion to estrogens. However, there is evidence that DHEA may have a specific receptor. This has been demonstrated in blood vessels, where DHEA binds with high affinity to nonhuman endothelial cell membranes without being displaced by structurally related steroids.

The present data seem to indicate that the effect of DHEA administration may not be entirely ascribed to the conversion in estrogenic metabolites. In fact, the E2 levels reached in DHEA-treated ovx animals were not significantly different from those observed in untreated ovx animals and significantly lower than those obtained in ovx animals in response to E2 valerate administration. Similar levels of β-EP and allopregnanolone, obtained in various organs, were observed in response to the two different therapies. Therefore, DHEA may act directly or through its local metabolites on neurosteroidogenesis and the opioidergic pathway. (83)

6. EFFECT OF DHEA THERAPY ON SEXUAL FUNCTION USING AN *IN-VIVO* MODEL

The field of behavioural pharmacology is showing an increasing interest in female rat sexual behaviour as a model for evaluating drug actions and steroid interference. Endogenous sex steroids modify female sexual behaviour in rats. Fertile animals during the estrous phase show normal level of sexual receptivity, which is abolished by ovariectomy and it can be partially restored by the subcutaneous injections of Estradiol Benzoate (EB) and Progesterone (P) (84). Sex steroids influence the neurobiology of sexual function, acting directly on their receptors at nuclear and membrane level or indirectly throughout their effects on neuropeptides (oxytocin, beta-endorphin, ecc.), neurotransmitters (dopamine, serotonin) and neurosteroid metabolism (mainly allopregnanolone) (85-86).

Sexual behaviour in the female rat is characterized by both receptive and proceptive behaviours (87). Receptive behaviour consists in a reflexive posture, called lordosis, which represents the female readiness to allow copulation (87). Proceptive behaviours, including hops, darts and ear-wigglins, are exhibited by a sexually receptive female to arouse male sexual interest (88). Consequently they are considered appetitive behaviours, while lordosis represents the consummatory aspect of female sexual behavior (89). Sexual motivation of female rats can be also assessed by evaluating their preference for a sexually active male versus a sexually receptive female, during the partner preference test (90,91). In addition, if the mating occurs in a laboratory condition which allows the female to enter and exit from the male's compartment, the estrous female will approach and withdraw from the male, controlling hence the number and the timing of sexual contacts (i.e. mount, intromission and ejaculation). This pattern, known as paced mating behaviour is thought to be influenced by sexual motivation besides to sensory discrimination between the different types of sexual stimulation (mount, intromission and ejaculation) (92).

In the present study, we evaluated the influence of DHEA administration on receptive and proceptive components of female rat sexual behaviour and whether the co-administration of estrogens might enhance sexual response in a model of ovariectomized rats.

6.1 Methods

Animals

Adult female Sprague-Dawley rats weighing 200-250 g were obtained from Charles River Laboratories (Calco, LC, Italy). They were housed two per cage and maintained in standard conditions at 22±1°C and 55-60% humidity. A 12-h reversed light/dark cycle was employed to facilitate behavioural testing during the normally active (dark) phase of the cycle. Commercial rat

pellets and water were freely available. After a week of adaptation, all rats were bilaterally ovariectomized under ketamine hydrochloride (Ketavet 100®, Farmaceutici Gellini Spa, Italy) plus xylazine hydrochloride (Rompun®, Bayer, Germany) anesthesia, using standard surgical procedures.

Sexually experienced Sprague Dawley male rats, weighing approximately 350-400 g, were used as stimulus animals in the behavioural tests.

Animal care, maintenance and surgery were conducted in accordance with the Italian law (D.L. n. 116/1992) and European legislation (EEC n. 86/609). The experimental design and procedures received the approval of the Bioethical Committee of the Italian Institute of Health.

Treatments

Treatments were started approximately 3 weeks after ovariectomy to allow a complete recovery from surgery. Animals were divided in 6 groups and submitted to the following treatments:

1) Estradiol Benzoate (Estradiol benzoate, Sigma-Aldrich, Milan, Italy) (EB) 10 μg/rat subcutaneously (s.c.) injected 48 h before the behavioural test;

2) Estradiol Benzoate (EB) 3 μg/rat s.c. injected 48 h prior to behavioural test;

3) DHEA (dehydroepiandrosterone, Sigma-Aldrich, Milan, Italy) 0.5 mg/kg by oral gavage (p.os) daily for 6 weeks;

4) DHEA 5 mg/kg p.os daily for 6 weeks;

5) EB 3 μg /rat s.c. 48 h prior to behavioural test plus DHEA dosed at 0.5 mg/kg p.os daily for 6 weeks;

6) EB 3 μg/rat 48 h prior to behavioral test plus DHEA dosed at 5 mg/kg p.os daily for 6 weeks.

All the females were s.c. injected with 500 μg of progesterone (Prontogest®, AMSA) 4 h before the tests. Hormones were delivered in a peanut oil vehicle. DHEA was solubilised in Tween 80 (10%) and water and administered in the volume of 5 ml/kg.

Sexual behaviour testing

1) Receptivity test

The test was conducted in a clear Plexiglas arena (70×35×40 cm high), where a male rat was placed for a 5-min period and was allowed two intromissions with a stimulus non-experimental female to

ensure his sexual vigor. Thereafter, an experimental female rat was placed in the arena with the male. The test ended when the female received 10 mounts with or without intromission. The lordosis response was scored on a four-point scale (0-3) as described (93). For each rat a lordosis quotient (LQ) was calculated as the number of lordosis responses (scores of 2 or 3) divided by the total number of mounts multiplied by 100. During the test the number of proceptive behaviours (hops-darts, ear-wigglings, crawlings, approach and withdrawal from the male and sniffings) and rejective behaviours exhibited per minute were recorded. The behavioural parameters were scored by observers unaware of the pharmacological treatments.

2) Partner preference test

After 5 weeks of treatment the partner preference test, performed according to Ågmo et al. (94), was used to evaluate sexual motivation in a no-contact condition. The apparatus consisted of an open field arena (100×50×40 cm high) with two round cages made of wire meshing (16 cm diameter, 40 cm high) diagonally positioned at the opposite corners of the arena. In the two cages a sexually active male and a female rat were individually placed as stimulus animals. A virtual area near to each animal cage was defined as the incentive zone: the sexual incentive area near to the male and the social incentive area near to the female. In these conditions it was allowed the transmission of visual, olfactory and auditory cues but it was avoided mating. Experimental females were individually placed in the centre of arena for a 5-min adaptation period at the presence of the stimulus animals and thereafter tested for 10 min. The number of visits to the male and the female as well as the time spent near each stimulus animal were recorded. The measure of sexual motivation is expressed by a preference score that is the ratio between the time spent in the sexual incentive area versus the total time spent in the two incentive areas (94).

3) Paced mating behaviour

After 6 weeks of treatment the experimental female rats were tested for paced mating behaviour according to the procedure described by Clark et al. (95). The apparatus consisted in a plexiglas arena (80×49×40 cm high) divided into three equal compartments by two clear plexiglas partitions (48.5×39.5 cm high) with holes (5 cm in diameter, placed 1.25 cm from the side and bottom edges) in the bottom corners. The small diameter of the holes enabled the female rat to enter and exit from the male compartment, while not allowing the access of male rat to the female's compartment. Two opaque partitions (48.5×39.5 cm high), covering the holes in the plexiglas ones, were used to avoid the female rat and the males to see one another, when they were not being tested.

The mating test started by placing the experimental female in the central compartment and a stimulus male rat in each of the two side chambers. The female rat was allowed to access to a single male rat through the hole of the clear plexiglas partition, after the removal of the opaque divider. The mating test ended when the female received 10 mounts with intromission. If ejaculation occurred, she was allowed to leave the male rat's compartment and then return. Thereafter the female was confined in the central compartment replacing the opaque partition. To complete the test the female rat was permitted to access to the second male rat. Paced mating test was terminated: (i) if the female returned to the stimulus male rat, following the 10^{th} intromission; (ii) if the female received no intromission within the first 15 min from the beginning of the test; (iii) when the interval between two intromissions was longer than 30 min. During the test the following parameters of sexual behaviour were registered or calculated: a) the contact-return latency, that is the time between the female's exit from male's compartment following each type of sexual stimulation (mount, intromission, ejaculation) and her return; b) the percentage of exits, that is the number of times the female rat left the male's compartment, following each type of stimulation.

Biochemical assays

Following the conclusion of all behavioural experiments, specifically 24 h after the paced mating test, female rats were sacrificed by decapitation and trunk blood collected. Brains and adrenal glands were removed for the subsequent biochemical analysis. From the brains the following areas were isolated: hypothalamus, hippocampus, anterior pituitary, neurointermediate lobe, frontal and parietal cortex.

All hormonal determinations were carried out during the same assay. Plasma DHEA, Progesterone (P), Estradiol (E2) Testosterone (T) concentrations were determined using commercially available radioimmunoassays kits (Radim, Pomezia, Rome, Italy). The intra-assay and inter-assay coefficients of variation (CV) and the sensitivity of the assay were: 7.8 and 8.3% and 0.02 ng/ml for DHEA, 8,9% and 0,03 ng/ml for T, 11.7% and 0.12 ng/ml for P, 8.5% and 4.7 pg/ml for E2, respectively. Serum corticosterone (ICN Biomedicals Inc., Irvine, CA, USA) was assayed by radioimmunoassay using trade kits. For corticosterone the assay sensitivity was 25 pg/ml and the intra- and inter-assay coefficients of variation were 5.8% and 7.5% respectively.

Allopregnanolone assay

The supernatant of tissue homogenates and serum was passed through a C-18 Sep-Pak cartridge, previously equilibrated with homogenizing buffer. The cartridge was sequentially washed with homogenizing buffer, 50% aqueous methanol, and the unconjugated steroid fraction was eluted with absolute methanol and brought to dryness under nitrogen. Analytical grade solvents were purchased from Merck (Darmstadt, Germany); C-18 Sep-Pak cartridge were obtained form Waters Corporation (Milford, USA). Allopregnanolone contents were measured by a radioimmunoassay method, using an antiserum kindly provided by Dr. RH Purdy (San Diego, CA, USA). The sensitivity of this assay was 20 pg/mL, the recovery after extraction and chromatography was 86.51±2.7% (mean ± SEM), and the intra- and inter-assay coefficient of variation was 7% and 9%, respectively. Allopregnanolone levels were expressed in pg/mg of tissue in each tissue and in pg/ml in serum.

β-Endorphin assay

The supernatant of tissue homogenates and plasma were passed through a C-18 Sep-Pak cartridge, previously equilibrated with 50% aqueous methanol, and the unconjugated fraction was eluted with absolute methanol and brought to dryness under vacuum. β-endorphin levels were measured by a previously described specific radioimmunoassay (83), using camel β-endorphin as standard (Sigma Chemicals, St. Louis, MO, USA). The antiserum was used at the final dilution of 1:130.000. Analytical grade solvents were purchased from Merck (Darmstadt, Germany); C-18 Sep-Pak cartridges were obtained from Waters Corporation (Milford, USA). The sensitivity of this assay was 10 pg/ml, the recovery following acetic acid extraction and chromatography corresponded to 85±11% of the total amount, and the intra- and inter-assay coefficients of variation were 6% and 8%, respectively. β-endorphin levels were expressed in ng/organ (hypothalamus, hippocampus and neurointermediate lobe) or ng/mg tissue (frontal and parietal cortex) and in ng/ml in plasm

Statistical Analysis

Behavioral and hormonal data, reported as mean ± SEM, were analyzed using GraphPad Prism Software, version 5.0 for Windows (GraphPad Software, San Diego, CA, USA). One-way analysis of variance (ANOVA) with Newman–Keuls test for post-hoc comparisons was performed between individual treatment groups. Values of $P < 0.05$ indicate significant differences among groups.

6.2 Results

Hormonal evaluation

-Effects of Estradiol Benzoate (EB 3-10 μg/rat), of DHEA (0,5-5 mg/kg) and co-administration of DHEA (0,5-5 mg/kg) plus EB (3 μg/rat) on Allopregnanolone (Allo) concentration in selective brain areas and in adrenal gland of ovariectomized rats.

In all brain areas analyzed, with the only exception of the frontal lobe, the administration of EB at 10 μg/rat in ovariectomized rats increased Allo content in comparison with EB 3 μg/rat. The administration of DHEA at the lower dose (0,5 mg/kg) did not evidence any significant difference in comparison with EB 3 μg/rat in all brain areas. On the contrary, DHEA 5 mg/kg increased Allo levels in comparison with EB at 3 μg/rat in all CNS areas evaluated. The co-administration of DHEA 0,5 mg with EB did not affect Allo level in all brain areas, in comparison with EB alone and with DHEA at 0,5 mg/kg. On the contrary, the co-administration of EB with the highest dose of DHEA (5 mg/kg) increased Allo levels respect to EB 3 alone in all brain areas and also in comparison with DHEA alone, at the dose of 5 mg/kg, in the parietal cortex, in the hypothalamus and in the anterior pituitary. In the adrenal gland, only the co-administration of DHEA (0,5 mg/kg and 5 mg/kg) with EB 3 increased Allo content in comparison with EB 3 alone, whereas all other hormonal treatments did not affect its intra-glandular concentration.

-Effects of Estradiol Benzoate (EB 3-10 μg/rat), of DHEA (0,5-5 mg/kg) and co-administration of DHEA (0,5-5 mg/kg) plus EB (3μg/rat) on Beta-endorphin (β-end) concentration in selective brain areas.

In all brain areas analyzed, with the only exception of the frontal lobe, EB, when administered at the dose of 10 μg/rat increased β-end content respect to EB at 3 μg/rat. The administration of DHEA alone at the lower dose (0,5 mg/kg) did not evidence any significant differences in comparison with EB 3 μg/rat in all brain areas. DHEA treatment at the dose of 5 mg/kg increased β-end levels in comparison with DHEA 0,5 mg/kg and in comparison with EB 3 μg/rat in hippocampus, in the hypothalamus and in the neurointermediate lobe. The co-administration of DHEA 0,5 mg with EB did not modify β-end level in all brain areas analyzed. On the contrary, the co-administration of EB with the highest dose of DHEA (5 mg/kg) increased β-end respect to EB 3 μg/rat alone in all brain areas and also in comparison with DHEA alone at the dose of 5 mg/kg in the hypothalamus.

-Effects of Estradiol Benzoate (EB 3-10 µg/rat), of DHEA (0,5- 5 mg/kg) and co-administration of DHEA (0,5-5 mg/kg) plus EB (3µg/rat) on circulating hormonal levels.

Female rats receiving EB 10 µg/rat, DHEA 5 mg/kg and the co-administration of DHEA + EB (5 mg/kg + 3 µg/rat) showed an increased levels of circulating estradiol in comparison with EB 3 µg/rat. Administration of DHEA at 0,5 mg/kg alone showed reduced levels of estradiol compared to EB 3, whereas no modifications on estrogen levels were found following the co-administration of EB + DHEA 0,5 in comparison with EB 3.

Plasma level of DHEA was found increased following the administration of both doses of DHEA alone or in co- administration with EB, evidencing a dose-related increase, although the presence of EB did not modify plasma level of DHEA.

Only animals receiving DHEA alone or in combination with EB showed increased level of testosterone in comparison with EB 3 or EB 10. In these animals, T level were not affected by the dose of EB, while the concentration of T were higher in rats treated with DHEA 5 in comparison with those receiving DHEA at the dose of 0,5 mg/kg.

Progesterone and corticosterone circulating levels were not modified following the administration of any dose of EB, DHEA or their co-administration.

Allo levels resulted significantly increased following treatments with EB 10 and with the co-administration of DHEA 5 + EB 3 in comparison with EB 3 alone. DHEA therapy alone, at 0,5 mg/kg and 5 mg/kg, did not affect the concentration of this steroid compared to EB 3.

Beta-endorphin circulating levels were increased only following the administration of the EB 10, DHEA 5 mg and the combination of DHEA 5 + EB 3 in comparison with EB 3 alone.

Behavioural tests

1) Receptivity test

The oral administration of DHEA alone at both dosages failed to induce receptive behaviour in female rats, even if they were treated with progesterone; therefore their LQ values were always undetectable over the 4 weeks (data not shown). Rats treated with EB dosed at 10 µg/rat exhibited high level of sexual receptivity (LQ= 68.6 after 1 week of treatment, 93.8 after 2 weeks, 97.5 after 3 weeks and 90.0 after 4 weeks) (Fig.1). Rats treated with EB dosed at 3 µg/rat showed a lower level of sexual receptivity in comparison with EB 10 µg/rat treated rats, except for the first week test

(LQ= 71.4 after 1 week of treatment, 77.5 after 2 weeks, 73.8 after 3 weeks, 81.4 after 4 weeks) (Fig.1). Female rats treated with EB 3 µg/rat and DHEA 0.5 mg/kg displayed LQ values which were not statistically different from those of EB treated rats at the same dosage (3 µg/rat). The administration of DHEA dosed at 5 mg/kg in EB treated rats induced a significant increase in LQ value in comparison with EB alone treated rats, only after 1 week of treatment ($p<0.05$) (Fig.1). It must be underlined that LQ value was significantly higher than that of EB 10 treated rats ($p<0.05$) (Fig.1). In the following tests performed after 2, 3 and 4 weeks the difference in LQ values between EB + DHEA and EB alone treated rats was not statistically different (Fig.1). As concerns proceptive behaviours detected during the test, female rats treated with EB and DHEA at both dosages exhibited a marked increase in comparison with EB (3 µg/rat) treated rats already after the first week of treatment ($p<0.05$) (Fig. 2). Starting from the second week the difference between EB+DHEA and EB alone treated rats was statistically significant only when the highest dose of DHEA was administered ($p<0.05$ after 2 weeks, $p<0.001$ after 3 and 4 weeks) (Fig.2). It must be underlined that the number of proceptive behaviours exhibited by DHEA 5 mg/kg + EB 3 µg/rat was higher than that of EB 10 µg treated rats, at any tested time (Fig.2). No difference was detected in the number of rejective behaviours among the different experimental groups (data not shown).

2) Partner preference test

As expected, rats submitted to EB treatment at both dosages showed a marked preference to visit the male rat rather than the female ($p<0.05$) (Fig.3 panel A). Consequently the preference score of EB treated rats was 0.77 following the injection of 10 µg/rat and 0.70 following the injection of the lower dose (3 µg/rat) (Fig.3 panel B). Female rats treated with DHEA alone failed to show a preference for the sexual or the social stimulus (Fig.3 panel A): therefore the preference score of both treated groups was approximately 0.50 (Fig.3 panel B). Rats administered with DHEA at both dosages and EB (3 µg/rat) exhibited a significant preference for the male rat (Fig.3 panel A): the preference score was found to be 0.73 in both treated groups (Fig.3 panel B).

3) Paced mating behaviour

Paced mating test was performed only in 3 groups of rats treated as follows: 1) EB 10 µg/rat; 2) EB 3 µg/rat; 3) EB 3 µg/rat + DHEA 5 mg/kg.

As shown in Fig.4, there was a significant main effect of type of sexual stimulation on contact-return latencies (mount<intromission<ejaculation). It must be stressed that the mount, intromission and ejaculation contact return latencies of EB 3 + DHEA 5 mg/kg treated females were lower than that of EB (3 µg/rat) treated rats: the difference was found statistically significant as regards the

latency to return to male's compartment after ejaculation ($p<0.05$) (Fig.4). For percentages of exits, there was a significant main effect of type of sexual stimulation but not a main effect of treatment (data not shown).

7.3 Conclusions

Sex steroids directly modify sexual function, evidenced as changes in sexual behaviour and in variations in the neurobiology of neuropetides and neurosteroids involved in sexual function.

It's well established by previous studies that ovariectomy reduces female brain content of allo and beta-endorphin in the hypothalamus and in sovra-hypothalamic areas and that two-weeks treatment with estrogens or DHEAS were able to increase the level of endogenous opioid and neurosteroidogenesis in a dose-related response. (83)

The present study confirms that chronic treatment (six weeks) of ovariectomized rats with DHEA only at the dose of 5 mg/kg increases allopregnanolone and beta-endophin content in different brain areas and in plasma when compared with available reference values of ovariectomized rats (16). Similarly, circulating hormonal changes after treatment with both doses of DHEA results in physiological increase of plasma estradiol, within the available range values of fertile animals (16) and no significant change of adrenal function (corticosterone level) were observed after treatment. These results, together with the available previous data, confirm that these animals received doses of DHEA inducing a biological response in a physiological range.

A single injection of EB at the higher dose (10 μg/rat) increased allopregnanolone and beta-endorphin brain and circulating content, supporting the concept that both systems are positively and directly influenced by estrogens in a dose-related way. Interestingly, co-administration of EB and DHEA showed a synergic effect on brain neurosteroidogenesis and opioid content compared to single treatments, although EB and DHEA therapies had a different time-schedule. In particular, this effect was shown over different brain areas and it was evidenced comparing animals receiving EB (3 μg/rat) and DHEA (5 mg/kg) as single therapy with rats receiving the combination of both.

Opioid receptor agonists infused into the lateral ventricles can inhibit (through mu receptors) or facilitate (through delta receptors) the lordosis behavior of ovariectomized (OVX) rats treated with estrogen and a low dose of progesterone.

The sexual effects of beta-endorphin are reputed to occur mainly through their action on the pre-optic area and the amigdala (101). Beta-endorphin infused into the medial pre-optic area inhibits mounting and intromission; infusion of this peptide into the medial amygdala inhibits the initial

appetitive phase (101-102). However, according to Argiolas (103) the inhibitory effect is dose dependent, with low doses of opiate having facilitatory effects and with high doses inhibitory ones and beta-endorphin may facilitate appetitive behaviour by acting on the ventral tegmental area to activate the mesolimbic dopaminergic system.

On the contrary, brain infusion of Allo positively affects all aspects of socio-sexual activities, enhancing exploratory, anti-anxiety and social function, (85). In particular, Allo plays a critical role in promoting sexual function in female rats: midbrain levels of this neurosteroid directly correlate with the onset and duration of lordosis and mating behaviour (85).

Although DHEA is not a main hormone in rats, it can be metabolized to estrogens and delta-4 androgens in different rat peripheral tissues and in brain by the widespread distribution of 17beta-HSD (104). However, the increase of circulation testosterone levels in rats receiving only DHEA did not induce any significant changes in sexual behavior. On the contrary, when animals received the conditioning treatment with EB, DHEA therapy amplified measures of sexual motivation. In addition, DHEA influence on brain function is multifaceted and it is not only a brain pre-hormone for estrogens and testosterone synthesis: DHEA acts as a modulator of neurotransmitter receptors, such as gamma-aminobutyric-acid-type A, N-methyl-D-aspartate, sigma subtype 1 (σ1) receptor, and glycine receptor, in addition to opioids and allopregnanolone. (104)

These brain specific effects of DHEA seems to be important for sexual function, since DHEA, but not its conversion to testosterone, alleviates the suppressive effects of social stress on copulatory behavior via σ1 receptors in male rats. (102)

It can be assumed that chronic treatment with DHEA might enhance or balance the biological response of certain brain circuits to the acute administration of EB, with neurochemical, hormonal and behavioral consequences, especially for sexual function.

Indeed, the present study on female sexual behaviour shows that the administration of DHEA in EB treated rats is able to enhance receptivity only after 1 week of treatment, while significantly increasing proceptive behaviours at any tested time. Proceptivity values were always found higher than that detected in EB 10 μg treated rats. There is a general agreement that an increased frequency of proceptive behaviours could be indicative of an increased sexual motivation (105).

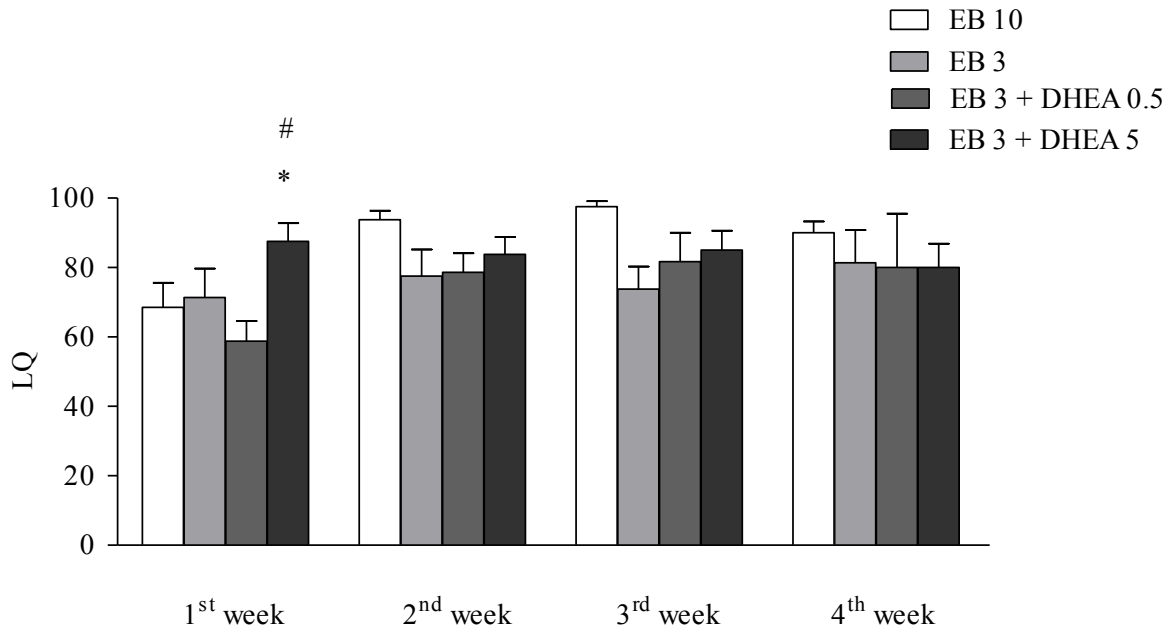
In the partner preference test, the preference score of EB +DHEA treated rats (0.73) was slightly higher than that of EB 3μ treated rats (0.70) but lower than that of EB 10 μ treated rats (0.77). The differences in the time and in the number of visits among EB + DHEA treated rats and EB 3μg

treated ones were not statistically significant. This finding is apparently in contrast with the observed increase in proceptivity during the receptivity test. It was suggested that the preference for a sexually appropriate partner is an index of the female sexual motivation and it is dependent on distal cues of the partner (auditory, visual and olfactory) rather than on coital stimulation (105). However it must be pointed out that the sexual motivation was tested during the receptivity test in a contact condition, while during the partner preference it was tested in a no-contact condition. Therefore DHEA seems to affect sexual motivation more markedly when physical contact was unrestricted (contact condition) than when physical contact was restricted (no-contact condition).

Also the results obtained in the paced mating test might suggest an increased motivation as indicated by reduced contact return latencies corresponding to a lower time spent by the female in her own compartment. While the contact return latency is thought to be a measure of sexual motivation, the percentage of exits is thought to be a measure of sensory discrimination. Therefore the lack of effect of DHEA on the percentage of exits suggests a direct central effect on motivation rather than an influence on the peripheral sensitivity.

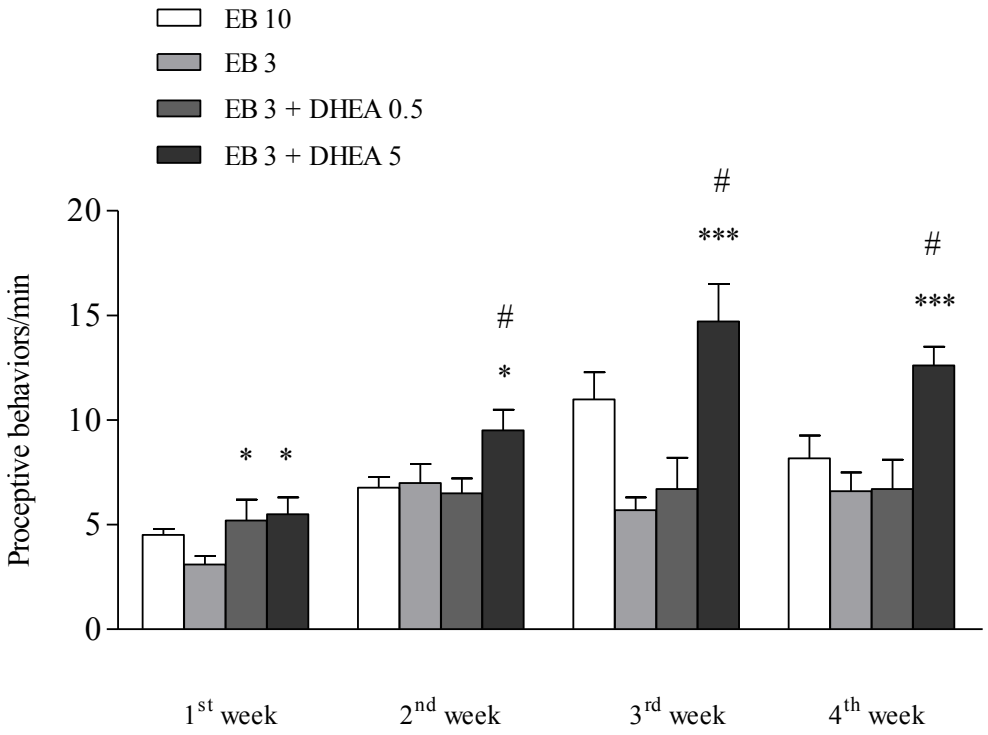
Taken together these results support the positive effect of DHEA treatment on central aspects of sexual function with particular regard to motivation, at least in a model of female rats.

Fig.1



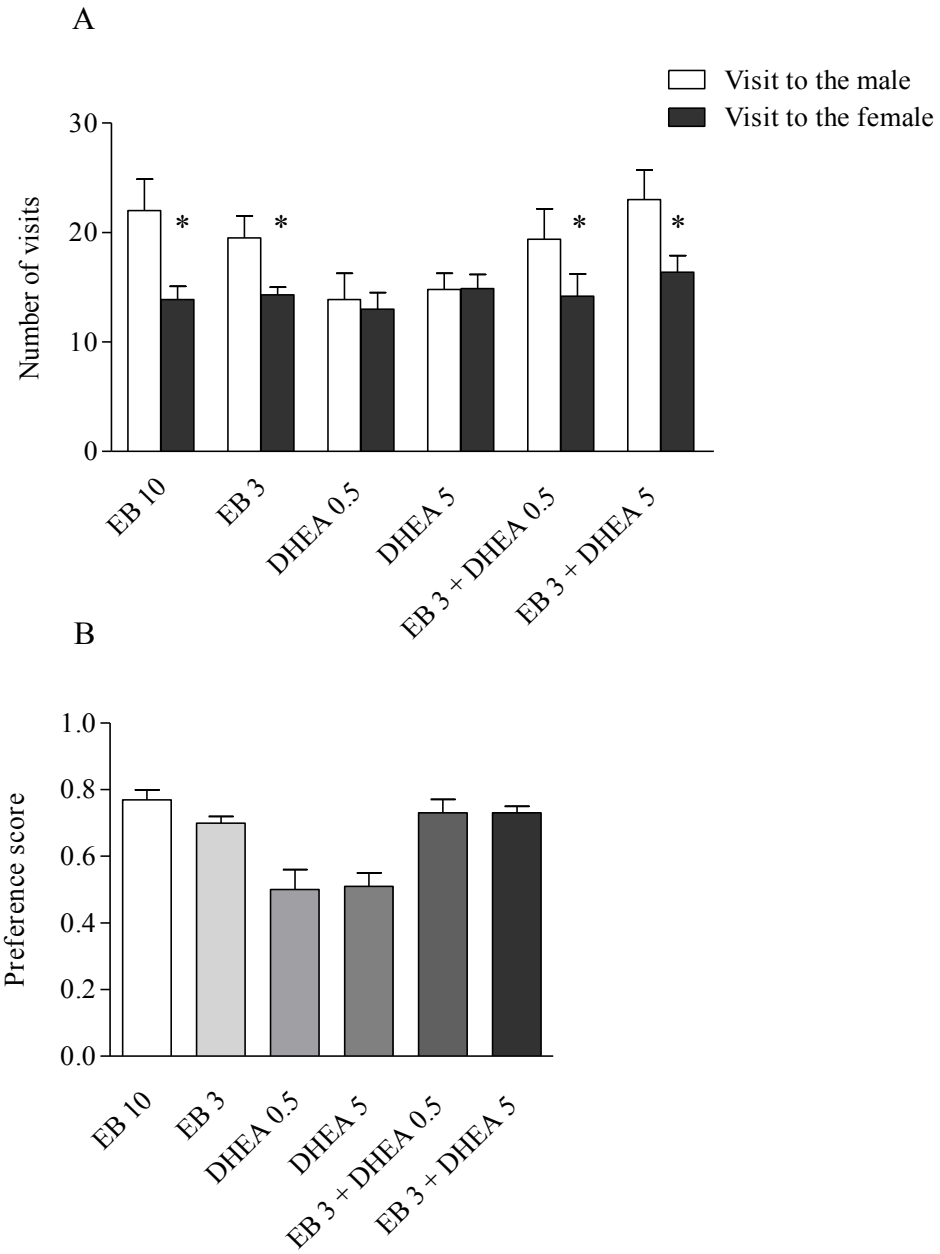
Effect of estradiol benzoate (EB), dosed at 3 and 10 µg/rat, and DHEA dosed at 0.5 and 5 mg/kg coadministered with EB (3µg/rat) on lordosis quotient (LQ) in ovariectomized rats after 1, 2, 3 and 4 weeks of treatment. Progesterone was injected 4 h before the test in all experimental rats. Values are mean ± SEM, n = 8. One-way ANOVA followed by Newman-Keuls post-test: $^{\#}P<0.05$ vs EB 10; $^{*}P<0.05$ vs EB 3 treated groups.

Fig. 2



Proceptive behaviors exhibited by ovariectomized rats treated with estradiol benzoate (EB) dosed at 3 and 10 μg/rat, and DHEA at the doses of 0.5 and 5 mg/kg coadministered with EB 3μg/rat, during the receptivity test performed after 1, 2, 3 and 4 weeks of treatment. Progesterone was injected 4 h before the test in all experimental rats. Values are mean ± SEM, n = 8. One way ANOVA followed by Newman-Keuls post-test: $^{\#}P<0.05$ vs EB 10, $^{*}P<0.05$, $^{***}P<0.001$ vs EB 3 treated groups.

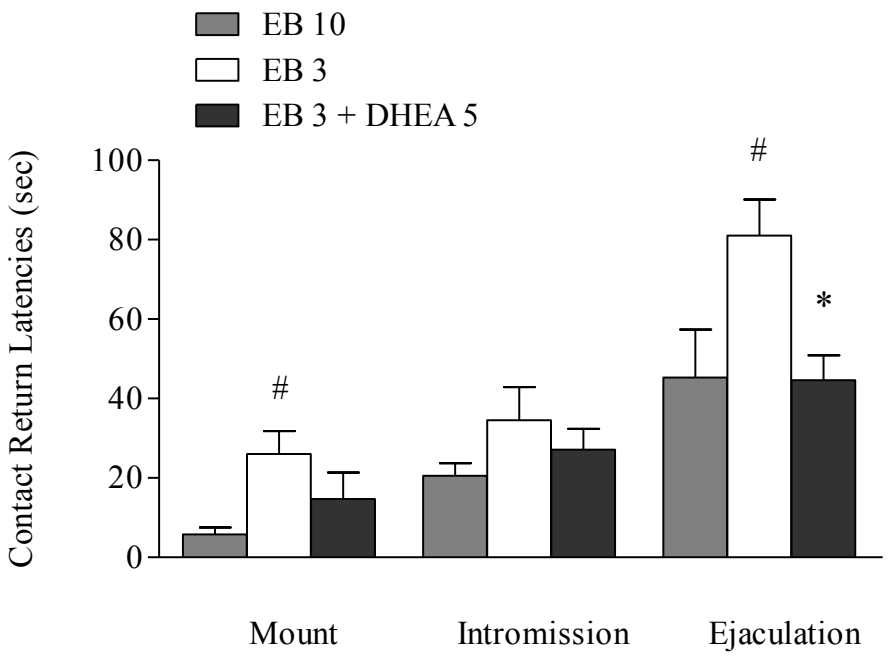
Fig. 3



Number of visits to the male and the female stimulus animals (panel A) and preference score (panel B) exhibited by ovariectomized rats treated with estradiol benzoate (EB) dosed at 3 and 10 μg/rat and DHEA, coadministered with EB 3 μg/rat or alone, at the doses of 0.5 and 5 mg/kg, during the partner preference test performed after 5 weeks of treatment. Progesterone was injected 4 h before

the test in all experimental rats. Values are mean ± SEM, n = 8. One way ANOVA followed by Newman-Keuls post-test: **P*<0.05 vs visits to the male.

Fig. 4



Contact return latencies exhibited by ovariectomized rats treated with estradiol benzoate (EB) dosed at 3 and 10 µg/rat and DHEA 5 mg/kg coadministered with EB 3 µg/rat, during the paced mating test performed after 6 weeks of treatment. Progesterone was injected 4 h before the test in all experimental rats. Values are mean ± SEM, n = 8. One way ANOVA followed by Newman-Keuls post-test: $^{\#}P$<0.05 vs EB 10, $^{*}P$<0.05 vs EB 3 treated rats.

7. DHEA EFFECTS ON CLINICAL MEASURES OF DEPRESSION, ANXIETY AND QUALITY OF LIFE IN WOMEN

Unlike DHEA(S) concentrations that decline under conditions of chronic stress and medical illness, cortisol concentrations generally either rise or do not change, and subsequently result in a decrease in DHEA(S)-to-cortisol ratios (106-109) As described previously, DHEA(S) and cortisol have different and often antagonistic effects on each other. The significance of considering DHEA(S)-to-cortisol ratios is exemplified by the concept of "anabolic balance," which considers the ratio of anabolic to catabolic hormones and may indicate susceptibility to diseases of stress and aging (110). Hormones co-regulate each other, and together, co-elevations or imbalances determine net effects on tissues. Therefore, it may be important to consider the ratio of both steroids in addition to their absolute concentrations. For example, greater cognitive deterioration was observed in elderly men and women who showed larger decreases in plasma DHEAS-to-cortisol ratios over a two-year period, although changes in DHEAS concentrations alone were not significantly correlated with cognitive change (111). Frail, institutionalized elderly people did not differ from independent community-dwelling controls in serum concentrations of cortisol or DHEAS, but did have significantly lower DHEAS-to-cortisol ratios (112).

Both DHEAS and cortisol are considered in the calculation of allostatic load, which is a measure of the cumulative physiological burden to the body of accommodating multiple stressors over time (113). Allostatic load scores are based on ten biological parameters, including DHEAS, cortisol, epinephrine, norepinephrine, high density lipoproteins (HDL), total cholesterol, waist-to-hip ratio, glycosylated hemoglobin (HbA1C), systolic blood pressure, and diastolic blood pressure. High cortisol concentrations and low DHEAS concentrations contribute to increases in allostatic load score. The hormonal profile contained within the measure of allostatic load by itself serves as a stronger predictor of cardiovascular disease than the traditional cardiovascular disease risk factors alone (114)

According to current theories of the biology of depression, DHEA(S)' ability to modulate many neurobiological actions, including glutamate and σ_1 receptors, catecholamines, neurogenesis, neuroprotection, anti-glucocorticoid, anti-inflammatory and anti-oxidant properties could all underlie relationships between endogenous and/or exogenously-supplemented DHEA(S) concentrations and depression. An assessment of depression ratings in relation to plasma concentrations of several steroid hormones (estradiol, testosterone, estrone, androstenedione, cortisol, DHEA, and DHEAS) in 699 non-estrogen using, community dwelling, postmenopausal women (aged 50–90 years) (115) found that only DHEAS concentrations were negatively correlated

with ratings of depressed mood. Specifically, higher DHEAS concentrations were associated with less depression, and this association was independent of age, physical activity and weight change. Furthermore, women with categorical diagnoses of depression had significantly lower plasma DHEAS concentrations compared to age-matched non-depressed women (115) Similarly, in a large-scale study of 2,855 well-functioning elderly men and women, serum DHEAS concentrations were inversely correlated with depressive symptoms. In a study looking at both DHEA and DHEAS in plasma, depressed patients had low DHEAS concentrations but normal DHEA concentrations. Women whose first onset of major or minor depression occurred during peri-menopause showed low morning plasma DHEA and DHEAS concentrations. Lower plasma DHEA concentrations during pregnancy and during the postpartum period were associated with higher postpartum ratings of depression. Dysthymic patients have also been shown to have low serum DHEAS concentrations. The remaining literature examining plasma and serum DHEA(S) concentrations in depression is inconsistent, with the decreased DHEA(S) findings described above, and with reports of either increased or unaltered DHEA(S) concentrations in depressed patients. A study examining diurnal salivary concentrations of DHEAS and cortisol in a small group of medicated but still depressed patients with unipolar depression, found that depressed patients had elevated DHEAS concentrations compared to controls.

Several groups have found that DHEA-to-cortisol ratios in serum and saliva, rather than concentrations of either hormone alone, more accurately discriminate depressed from non-depressed individuals (116) with lower morning ratios seen in depression (116. The molar DHEA-to-cortisol ratio was significantly lower in the un-medicated depressed patients than in controls, and the evening salivary DHEA-to-cortisol ratio ratios were inversely correlated with the lengths of current depressive episodes. Morning salivary DHEA hyposecretion as well as evening cortisol hypersecretion were significantly and independently associated with major depression in 8- to 16-year-olds. Patients who remained depressed several months after the initial assessment had lower salivary DHEA-to-cortisol ratios at baseline. Elevated DHEA(S) concentrations, relative to cortisol, may blunt the negative effects of high cortisol concentrations on depression.

The relationship between DHEA(S) concentrations and depression is complex. There is no parsimonious way of reconciling the diverse findings, but age of the subjects studied, demographic variables, comorbid psychiatric and medical diagnoses, acute vs. chronic stress, medication status and timing of the sample collection, are likely relevant. Gender may also have a significant impact. In a prospective study of a nationally representative sample, men with initially lower serum concentrations of DHEAS had greater increases in depression ratings over a three-year period; this relationship in men was not observed in women. Among African-American women (but not men),

lower serum DHEAS concentrations were associated with higher depression ratings. In another study, men with recurrent unipolar depression had low 24-hour urinary DHEA concentrations but normal cortisol concentrations, while women had normal DHEA concentrations but elevated cortisol concentrations. However, in both cases, the DHEA-to-cortisol ratios appeared to be low, suggesting that the anabolic balance may be a helpful way of identifying common states of general hormonal imbalances in certain clinical conditions.

7.1. Anxiety Spectrum Disorders

As reviewed above, DHEA(S) has prominent effects on $GABA_A$ receptor activity; these effects could be involved in the relationship between DHEA(S) and anxiety disorders. Increased plasma concentrations of DHEA were observed in male patients with panic disorder but not in females , and increased serum DHEAS-to-cortisol ratios were reported in a sample of patients of both genders who had panic disorder. Social anxiety disorder (also called social phobia), was not found to be associated with alterations in plasma DHEA(S) concentrations

The anxiety disorder that has received the greatest attention with regard to plasma, serum, and salivary DHEA(S) concentrations is post-traumatic stress disorder (PTSD) Studies have uniformly identified elevated DHEA and/or DHEAS concentrations in PTSD, as well as increases in the DHEA(S)-to-cortisol ratioUntreated men with combat-related PTSD were found to have increased plasma DHEA and DHEAS concentrations Another study found that PTSD patients who attempted suicide had increased plasma DHEA concentrations. Female victims of intimate partner violence with PTSD had increased evening salivary cortisol concentrations and increased morning and evening salivary DHEA concentrations compared to non-abused women . However, salivary DHEA concentrations were not significantly correlated with PTSD ratings. In a study of recently resettled refugees in Sweden, those with PTSD (but without depression) had elevated plasma DHEAS concentrations compared to refugees with neither PTSD nor depression. Over nine months of follow-up in these refugees, increases in PTSD symptoms were associated with increases in plasma DHEAS concentrations.

Despite the uniformity of studies showing elevations in DHEA or DHEAS in PTSD, researchers have suggested that the increase in DHEA(S) is salutary rather than pathophysiologic. Pre-menopausal women with chronic PTSD had increased plasma DHEA responses to ACTH stimulation compared to healthy, non-traumatized participants. In the women with PTSD, the peak change in plasma DHEA (in response to ACTH) was negatively correlated with PTSD symptoms, suggesting that increased capacity of adrenal DHEA release may mitigate the severity of PTSD symptoms. Consistent with that interpretation, another group found that, although plasma DHEA

and DHEAS concentrations were elevated in male veteran PTSD patients, concentrations of both hormones were directly correlated with symptom improvement and better coping. In another study, PTSD patients who responded to psychotherapy with a decrease in PTSD symptoms had an increase in DHEA concentrations in plasma, while patients who did not respond to psychotherapy had decreases in DHEA concentrations (after controlling for depressive symptoms).

Increased DHEAS concentrations under conditions of stress may indicate a salutary process. Although not in PTSD patients *per se*, a study of 19 men undergoing stressful military training including captivity exercises showed significant increases in both cortisol and DHEAS in saliva during the acute stress of training. Performance during a low intensity captivity challenge (but not during a high intensity one) was positively correlated with salivary DHEAS concentrations. In another study of 25 elite special operations soldiers exposed to prolonged and extreme training stress, soldiers experiencing fewer symptoms of dissociation and showing superior military performance had significantly higher ratios of plasma DHEAS-to-salivary cortisol. In light of such data, DHEA(S) has been proposed to play a role in resilience and in successful adaptation to stress (117-119).

Many, but not all, studies have reported lowered serum concentrations of DHEA(S) in patients with poor life satisfaction, psychosocial stress and functional limitations. Low plasma and serum concentrations of DHEAS have been associated with higher ratings of perceived stress, trait anxiety, as well as Type A behavior, cynicism, and hostility. Higher plasma and serum DHEAS concentrations have been associated with higher levels of functioning, higher likelihood of living independently and a lower likelihood of organic brain syndrome in men. Higher plasma and serum concentrations of DHEAS have also been associated with greater amount, frequency, and enjoyment of leisure activities, sexual gratification and frequency of masturbation (in women), healthier psychological profiles (90), more expansive personality ratings, and greater sensation-seeking and monotony-avoidance attributes. Most of these studies examined concentrations of DHEAS rather than DHEA, and many assessed female rather than male populations, so the generalizability of these findings is uncertain. In some studies, the relationships were gender-specific .

7.2 Effect of DHEA administration on depressive symptoms

Although clinical trials of DHEA treatment for depression are few in number, they consistently suggest beneficial effects. In an initial small-scale, open-label pilot study, DHEA treatment resulted in significant antidepressant effects in un-medicated middle-aged to elderly patients with major depression (121). The doses of DHEA were individually adjusted between 30 and 90 mg per day for four weeks to achieve circulating DHEA and DHEAS concentrations in the mid-to-high normal physiologic range for healthy young adults. Subjects demonstrated highly significant improvements in Hamilton Depression Ratings and Symptom Checklist-90 ratings. Mood improvements were significantly related to increases in the circulating concentrations of DHEA and DHEAS and to their ratios with cortisol; changes in cortisol concentrations alone were not correlated with behavioral changes (120) This small open-label study was followed by a double-blind, placebo-controlled trial in which 22 depressed patients received either DHEA (60 to 90 mg per day) or a placebo for 6 weeks (121). Some patients were medication-free at the time of entering the study; others remained depressed despite being on pre-stabilized (for a minimum of 6 weeks) antidepressant medication. In the former group, DHEA or the placebo was used alone; in the latter group, DHEA or the placebo was added to the stabilized antidepressant regimen. DHEA, compared to the placebo, was associated with significant antidepressant responses. Five of 11 DHEA-treated patients showed greater than 50% improvement in depression ratings and had endpoint Hamilton Depression Rating Scale ratings of less than 10, suggesting that they had responded to treatment. None of the 11 placebo-treated patients achieved these milestones (121). These results raised the possibility that DHEA, used alone or as an antidepressant adjunct in refractory patients, has significant antidepressant effects in some patients. Subsequently, another research group conducted a 12-week, double-blind, placebo-controlled study in un-medicated patients with mid-life dysthymia (one subject also had concurrent major depression) (39).

Second, the psychological symptoms of depression improved in both studies to a greater extent than the neurovegetative symptoms (e.g., sleep and appetite disturbances). Third, baseline serum DHEA concentrations did not predict antidepressant response, suggesting that DHEA supplementation was not simply correcting a DHEA deficiency in these patients (in which case, it would be expected to work only in those with low DHEA at baseline). Finally, responders to DHEA in both studies achieved higher serum DHEA concentrations following treatment than did non-responders, and antidepressant effects were directly correlated with changes in serum DHEA concentrations. This concordance across two separate studies in different populations strengthens the argument that the DHEA treatment itself is related to the antidepressant responses.

Thus, to date, every controlled trial of DHEA in depression has reported significant antidepressant effects. Although these data are encouraging, more large-scale studies will be required to establish the place, if any, of DHEA in the management of patients with depression. For example, there have been no head-to-head trials comparing DHEA to standard antidepressants, although in at least one trial, antidepressant non-responders did respond to DHEA augmentation. The risks and benefits of long-term DHEA administration also remain to be further clarified.

8. EFFECT OF DHEA ON SEXUAL FUNCTION IN WOMEN

Menopause is an individual experience that derives from a complex interplay of biological, psychological and relationship factors that collectively affect the physical, mental and sexual well-being of many women. Natural menopause is associated with a high rate of sexual symptoms. The reported prevalence of these varies and seems to depend on several factors, including the study sample (e.g. the women's socio-cultural background), the study design and the specific symptoms selected . Ageing and menopausal transition may in themselves impair the integrity of multiple biological systems involved in the normal sexual response. In addition, though, intrapersonal and interpersonal issues can affect feminine identity and sexual relationships. Inadequate hormone-dependent vulvo-vaginal receptivity (genital arousal, lubrication) is likely to cause sexual pain disorders, with profound consequences for sexual desire, mental arousal, orgasmic capacity and sexual satisfaction. Across the menopausal transition, the estradiol (E2) level is one of the best predictors of sexual function. Nonetheless, several domains of sexual response, such as sexual desire, sexual arousal and well-being, are commonly attributed to circulating androgens.

Taking into consideration that endogenous hormone levels may be poor indicators of their effects in target tissues, various studies have explored the relationships between androgens and clinical characteristics including sexual function, well-being, cardiovascular disease (CVD) risk, insulin resistance, and cognition. Relationships between androgens and sexual function were investigated in a cross-sectional study of 1423 non-health care-seeking women, aged 18 to 75 yr, randomly recruited from the community via the electoral roll, of whom 1021 completed a validated sexual function questionnaire (122). Androstenedione and total and free T were not related to sexual function scores. However, women aged 45 yr or more with low sexual responsiveness had a greater likelihood of having a serum DHEAS value below the 10th centile for their age (odds ratio (OR), 3.9;95%confidence interval (CI), 1.54–9.81; P 0.004). For women aged 18 to 44 yr, having low sexual desire, sexual arousal, or sexual responsiveness was also associated with having a DHEAS value below the 10th centile for their age (122). Because the normal range for serum DHEAS among young women is relatively large and a significant proportion of women with low DHEAS do not have low sexual function, a cutoff level below which women can be said to be more likely to have low sexual function cannot be identified. A smaller study of women attending a sexual medical center diagnosed with hypoactive sexual desire disorder, compared with controls, also reported an association between low DHEA and DHEAS and impaired sexual function (123).

Because it has been proposed that DHEA and DHEAS may exert neuroprotective effects, associations between endogenous DHEAS levels and cognitive performance in women aged 21 to

77 yr have been investigated. Women with higher levels of DHEA Sexhibited better performance on testing of executive function, with circulating DHEAS levels being significantly positively associated with higher scores for tests of simple concentration and working memory in women with at least 12 yr of education (124).

Exogenous DHEA Treatment

It has been proposed that treatment of postmenopausal women with DHEA will result in androgenic effects and hence improve libido and well-being via its conversion to T and estrogenic effects resulting in improvements in menopausal vasomotor symptoms. DHEA has been administered orally and parenterally, either by the transdermal or vaginal route. When administered to postmenopausal women, DHEA is mainly transformed to androgens rather than estrogens. Using liquid chromatography combined with tandem mass spectrometry, an oral dose of DHEA 50 mgdaily for 12 months (125) results in significant increases in estrone and estradiol, in the order of 34 and 57%, respectively, and a 200% increase in 5-diol. With this oral dose, total T levels increase by about 100%, serum DHT is relatively unchanged, and serum 3 -diol,3G, 3 -diol,17G, and ADT-G each increase 4- to 5-fold (125). These changes contrast with those reported in a 12-month study of transdermal DHEA using the same methodology for steroid measurements by the same laboratory (126). Irrespective of dose, with transdermal DHEA similar proportional increases in estrone, estradiol, and 5-diol were observed to those seen with oral DHEA, whereas the sum of ADT-G 3 -diol,3G and 3 -diol,17G increased by only 71% (126). Giagulli et al. and others have shown that hepatic 5 -reductase is a major determinant of the conversion of precursors to plasma ADT-G. This would be consistent with the mode of administration of DHEA influencing its metabolism, such that the higher ADT-G levels seen with oral DHEA reflects first past hepatic metabolism and not peripheral androgen formation.

First author, year (Ref.)	Study design	Duration (wk)	Dose (mg/d)	No. of PM women (age in yr)	Sexual function	Instrument to measure sexual function	Well-being	Instrument to measure well-being
Mortola, 1990 (9)	Placebo open label crossover	4	1600	6 (46–61)	No change	Self-reported	Not assessed	
Morales, 1994 (16)	DB placebo crossover	24	50	15 (8 on HT) (40–70)	No change	Visual Analog Scale	Not assessed	
Wolf, 1997 (70)	DB placebo crossover	2	50	15 (69 ± 1.7)	No change	Self-reported	Nonsignificant improvement in mood and wakefulness	QOL Mood questionnaire CESDS
Bloch, 1999 (77)	DB placebo crossover	6	90 oral (3 wk), 450 oral (3 wk)	3 (45–63)	Not assessed		Significant improvement in mood	BDI, HDRS, CDS
Baulieu, 2000 (71)	DB placebo parallel	52	50	140 (>60)	Improvement	Visual Analog Scale	Not assessed	
Hackbert, 2002 (72)	DB placebo crossover	1	300	16 (51–68)	Improvement	FES, DSFI, OFQ, self-report, vaginal photoplethysmograph	Not assessed	
Schmidt, 2005 (73)	DB placebo crossover	6	90–450	6	Improvement	DSFI	Significant improvement in mood	HDRS, BDI, CDS
Nair, 2006 (78)	DB placebo parallel	104		57 (>60)	Not assessed		No change	HSQ, SF-36
Kritz-Silverstein, 2008 (74)	DB placebo parallel	52	50	115 (55–85)	No change	Female Sexual Function Index	No change	BDI, SF-36, LSI-Z, SWLS
Labrie, 2009 (76)	DB placebo parallel	12	0.25, 0.5, 1.0% vaginal cream	218 (42–74)	Improvement	Abbreviated Sexual Function, MENQOL	No change	Psychological General Well-being Index
Panjari, 2009 (66)	DB placebo parallel	52	50	93 (40–65)	No change	Sabbatsberg Sexual Self-Rating Scale, sexual event diary, MENQOL	No change	Psychological General Well-being Index

DB, Double-blind; PM, postmenopausal; HT, hormone therapy; FES, Film Evaluation Scale; DSFI, Derogatis Sexual Functioning Inventory; OFQ, Orgasmic Functioning Questionnaire; MENQOL, Menopause-specific Quality of Life; BDI, Beck Depression Inventory; HDRS, Hamilton Depression Scale; CDS, Cornell Dysthymia Scale; SF-36, The Medical Outcomes Study 36-item Short Form Survey; LSI-Z, Life Satisfaction Index-Z; SWLS, Satisfaction with Life Scale; HSQ, Health Status Questionnaire; CESDS, Center for Epidemiologic Studies Depression Scale.

Studies of DHEA for Sexual Function

Although the prevalence, incidence, and antecedents of female sexual dysfunction remain under-researched, the most commonly reported sexual problems in women relate to sexual desire and interest, pleasure, and global satisfaction. To date, there are eight published randomized trials of oral DHEA treatment for low sexual function in healthy, postmenopausal women. These are summarized in Table below (125-134). Some of the studies demonstrated a positive effect of DHEA treatment on sexual function whereas others did not show any benefit. Of the three trials where a benefit was shown, two administered supraphysiological DHEA doses and were of short duration). The third study was of older aged women and employed a nonvalidated measure of sexual function that was understood by only 25% of the participants. The early studies in which DHEA was ineffective were also limited by small sample size, short treatment duration, use of nonvalidated instruments, or supraphysiological doses. More recent studies have employed validated measures of sexual function, have larger sample sizes, and are of longer duration. Two different studies with a 52-wk treatment phase have shown no improvement in sexual function with DHEA 50 mg daily. One was in 115 older, postmenopausal women, and sexual function was assessed by the Female Sexual Function Index. In the other, which excluded women with dyspareunia, sexual function was

assessed by two methods: a validated questionnaire and a 28-d diary of satisfactory sexual events . In contrast, the use of vaginal DHEA has been evaluated over 12 wk in women primarily presenting with dyspareunia and subjective vaginal dryness and irritation. Vaginal atrophy was reversed with minimal changes in serum steroid hormone levels, which remained within the normal postmenopausal range. Beneficial effects on four aspects of sexual dysfunction, desire/interest, arousal, orgasm, and pain at sexual activity were reported for this study. These data suggest that local combined androgenic/estrogenic stimulation in the vagina may exert favorable effects on sexual function in women suffering from vaginal atrophy. Unfortunately, the number of women allocated to each treatment group in this study was small, and the study reports do not provide information regarding the number of women in each arm that completed the study. Hence, the findings need to be reproduced in a larger study of longer duration before DHEA can be considered a therapeutic option for the management of vaginal atrophy. Whether these effects hold for women without vaginal atrophy remains to be investigated. Overall, the evidence from published RCT does not support efficacy of systemic DHEA therapy for the treatment of female sexual dysfunction. However, vaginal application of DHEA may benefit postmenopausal women with vaginal atrophy experiencing dyspareunia.

9. EFFECT OF 1-YEAR, LOW-DOSE DHEA THERAPY ON CLIMACTERIC SYMPTOMS AND FEMALE SEXUALITY

The effects of DHEA therapy on sexual function in postmenopausal women are controversial and there is still no conclusive evidence for its clinical use. On this basis, we aimed to evaluate the effects exerted by 1-year, low-dose oral DHEA therapy (10 mg/day) in symptomatic postmenopausal women on measures of sexual function and on hormonal changes in comparison with other three active treatments: daily oral continuous combined treatment with 1 mg micronized 17 β -estradiol plus 5 mg dydrogesterone or oral tibolone (2.5 mg/day) or vitamin D/calcium carbonate.

9.1 Methods

Department of Obstetrics and Gynecology, University of Pisa and from the Francavilla Fontana Hospital ‘ D. Camberlingo ’ , Italy, were enrolled in the study. All subjects had natural menopause and were healthy. Exclusion criteria for patient enrolment were: previous or current endocrine disorders, such as thyroid or adrenal dysfunction or altered prolactin circulating levels; treatment of cardiovascular diseases, hypertension or psychiatric disorders; previous or current hormone treatments known to influence endocrine function; smoking; presence of any kind of pelvic and breast disease. Natural menopause was defined retrospectively after 12 consecutive months without natural menstrual periods, and age at menopause was the age at last menstruation. The same physician followed each patient monthly. The Local Committees approved the study protocol and a written informed consent was obtained from each subject before the beginning of the study. An accurate medical, urogynecological and obstetric history was taken, including use of medications and lifestyle risk factors, as part of the sexual history, with the aim of identifying any possible organic factor affecting desire, arousal, orgasm and sexual pain. Validated tools (self-administered questionnaires) were used to properly diagnose sexual symptoms and to gain information on any sexual relationship. The self-administered questionnaire used was the McCoy Female Sexuality Questionnaire (MFSQ) (135). The MFSQ is a 19-item questionnaire developed to assess sexual interest, satisfaction with frequency of sexual activity, vaginal lubrication, orgasm, and sexual partner. It was designed to measure aspects of female sexuality likely to be affected by changing sex hormone levels. We used an Italian validated version (136). Women who reported climacteric symptoms and who requested hormone therapy were randomized uniformly into three groups and received the following treatments: the first group (n 12) received DHEA 10 mg daily, the second

group (n 12) were given daily oral estradiol (1 mg) plus dihydrogesterone (5 mg) (HRT) (Femoston Conti ® , Solvay Pharma SpA), the third group (n 12) received one daily oral tibolone tablet (2.5 mg) (Livial ® , Schering-Plough SpA). The fourth group (n 12) was formed from women who reported any climacteric symptoms and who refused hormone therapy; this group was treated with oral vitamin D (400 IU) plus calcium carbonate (1250 mg) (vitamin D group) to prevent osteoporosis. Randomization, in three hormone-treated groups, was made using a computer-generated block, random-permutation procedure. Compliance was checked by pill counts at monthly intervals. The study protocol was prospective and the treatment lasted for 12 months for all study subjects.

Main outcome measures

Each subject underwent a clinical and hormonal evaluation at baseline and at 3, 6 and 12 months of treatment. After overnight fasting, blood samples were obtained from each participant at 08.00 before the initiation of each hormone treatment in order to assess the levels of DHEA, DHEAS, Δ 4-androstenedione, testosterone, free testosterone, progesterone, estradiol, follicle stimulating hormone (FSH), luteinizing hormone (LH), sex hormone binding globulin (SHBG), cortisol, allopregnanolone, and β -endorphin. We also evaluated baseline levels of thyroid function. All blood samples were immediately centrifuged and stored at −20 ° C until required for the assay.

Each patient underwent an ultrasound pelvic evaluation to measure endometrial thickness and an ultrasound evaluation of bone density by Achille Insight (GE Healthcare) at baseline and after 12 months of treatment. All the patients completed the McCoy Questionnarie at baseline and before the end of study (after 12 months of treatment).

Assays

All hormonal determinations were carried out during the same assay. Plasma DHEA, DHEAS, Δ 4-androstenedione, testosterone, cortisol, progesterone, estradiol, and SHBG concentrations were determined using commercially availablradioimmunoassay kits (Radim, Pomezia, Rome, Italy). The intra-assay and interassay coefficients of variation (CV) and the sensitivities of the assay were: 7.8, 8.3% and 0.02 ng/ml for DHEA; 6.8, 8.5% and 0.02 ng/ml for DHEAS; 4.2, 7.6% and 0.03 ng/ml for Δ 4-androstenedione; 3.8, 8.7% and 0.017 ng/ml for testosterone; 6.2, 9.7% and 0.18 pg/ml for free testosterone; 4.9, 7.9% and 0.9 μ l/l for cortisol; 6.6, 11.7% and 0.12 ng/ml for progesterone; 4.6, 8.5% and 4.7 pg/ml for estradiol; 6.2, 6.2% and 0.18 mIU/ml for FSH; 7.8, 8.2% and 0.20 mIU/ml for LH; 3.8, 4.4% and 0.26 nmol/l for SHBG; respectively. Allopregnanolone evaluation was performed after extraction and chromatography partition on Sep-Pak C18 cartridges using our

previously used and described radioimmunoassay method (24). The sensitivity of the assay, expressed as the minimal amount of allopregnanolone distinguishable from zero, with 95% probability, was 20 pg/tube and the intra-assay and interassay CVs were 7.2 and 9.1%, respectively. The β -endorphin concentrations were determined after extraction and chromatography partition on Sep-Pak C18 cartridges, using a previously described radioimmunoassay method 25. The sensitivity of the assay was 2.5 pg/ml and the intra-assay and interassay CVs were 6.0 and 9.0%, respectively.

Statistical analysis

Data are expressed as mean standard error of the mean. Statistical analysis was carried out using Graph Pad Prism 4.0. Repeated measures one-way ANOVA, followed by a post-hoc analysis with Bonferroni multiple comparisons test, was used to evaluate differences in hormonal status and McCoy scores between the four groups. The α value for Bonferroni ' s test was set at 0.05.

9.2 Results

Patient characteristics All those patients who enrolled in the study completed the follow-up, without any adverse events. No significant differences were observed for chronological age, menopausal age and body mass index (BMI) between all four groups. The baseline endometrial thicknesses and bone densities (T –score calcaneal level) were similar in all groups (Table 1).

Endocrine evaluation

Baseline status

Before the start of treatment, there were no differences between the four groups regarding levels of LH (Figure 1), estradiol, DHEA, DHEAS, Δ 4-androstenedione, testosterone, free testosterone, progesterone, 17-hydroxyprogesterone, SHBG, allopregnanolone and β -endorphin. We observed a difference at baseline in FSH levels between the DHEA (90.8±9.1), HRT (69.5 ± 10.7) and vitamin D (69.7± 19.7) groups (p 0.05) (Figure 1), and in basal cortisol levels in the tibolone group (182.4 ± 15.2) vs. the DHEA group (204.5 ± 12.8) (p 0.01) and the vitamin D group (208.7 ± 15.8) (p 0.05).

Post-treatment evaluation

In women treated with DHEA, we observed a progressive significant increase in serum levels of DHEA (from 1.9 ± 0.6 to 2.7 ± 0.6 ng/ml) and of DHEAS (from 0.5 ± 0.1 to 0.8) 0.2 μ g/ml) during the entire treatment period, and an increase in Δ 4-androstenedione (from 0.9 0.1 to 1.28

0.2 ng/ml) (p 0.001) and testosterone (from 0.32 ± 0.12 to 0.54 ± 0.06 ng/ml) (p 0.05) levels from the 6th month, reaching the higher concentration at the 12th month. In the group treated with HRT, we observed no change in serum levels of DHEA, DHEAS, free testosterone and Δ 4-androstenedione during the entire treatment period and a progressive decline in testosterone levels (from 0.4 0.1 ng/ml from the 3rd month of therapy (p 0.001). In the group treated with tibolone, we observed no differences in androgen serum levels during the 12-month evaluation.

Estradiol levels showed a significant increase in all hormone-treated groups. In women treated with DHEA, we observed a progressive increase from 20.10 ± 3.2 to 27.5 ± 2.8 pg/ml; in the HRT group, we observed an increase from 18.1 ± 2.0 to 59.0± 9.0 pg/ml; in the tibolone group, there was an increase from 18.1 ± 2.1 to 23.8 ± 2.3 pg/ml (p 0.001) (Figure 2).

Progesterone levels showed a constant increase in the DHEA group (from 0.41 0.11 to 0.48 0.11 ng/ml) and the HRT group (from 0.42 ± 0.07 to 0.51 ± 0.07 ng/ml) (p 0.001). In women treated with DHEA, we observed an increase in progesterone levels and in 17-hydroxyprogesterone levels from the 6th month compared to baseline (p 0.001). In the group treated with HRT, as we expected, we observed a significant increase in progesterone and 17-hydroxyprogesterone levels from the 3rd month of treatment (p 0.05). In the tibolone group, we observed no change in progesterone and 17-hydroxyprogesterone levels during the entire treatment period.

Allopregnanolone levels showed a constant increase in all hormone-treated groups. In the DHEA group, we observed a progressive increase from the 6th month (from 198.2 ±18.0 to 223±17.1 pg/ml), and also in the tibolone group (from 197.3 ± 12.9 to 227.9 ± 18.8 pg/ml), and in the HRT group just from the 3rd month (from 210.1 ± 23.1 to 270.9± 35.5 pg/ml) (p 0.001).

Cortisol levels showed a significant reduction in all hormone-treated groups at the end of treatment (p 0.001). We observed a continuous increase in β -endorphin levels in all hormone-treated groups compared to the group given vitamin D and to baseline from the 3rd month. In the DHEA group, the β -endorphin level increased from 17.9 ± 3.0 to 35.3 ± 3.4 pg/ml; for the HRT group, it increased from 19.5 ± 2.3 to 41.2 ± 4.8 pg/ml; and for tibolone group, it increased from 17.2 4.1 to 23.9 ± 4.6 pg/ml (p 0.001).

SHBG levels increased slowly and progressively in the DHEA group from the 6th month and in the HRT group from the 3rd month of treatment (p 0.05). In women treated with tibolone, we observed, as expected, a decrease in SHBG, more significant after 12 months of therapy (p 0.001). No significant differences in SHBG levels resulted between the groups throughout the follow-up period (Table 2).

Climacteric symptoms

The severity of climacteric symptoms was analyzed using the Greene Climacteric Scale 26 at baseline and after the end of the active treatments. In particular, women receiving hormone treatments (DHEA, HRT or tibolone) had the same degree of climacteric syndrome at baseline, which was higher than in women receiving only vitamin D (15.1 ± 8.5). After 12 months, symptoms were improved in women treated with all hormone preparations, whereas no changes occurred in the group of women receiving vitamin D.

McCoy Female Sexuality Questionnaire

The four groups did not differ significantly with regard to baseline sexual function using the total score of the McCoy

Female Sexuality Questionnaire.

Before any treatments, in regard to the relationship total score, we observed a good baseline value in all the groups (Figure 1). As regard frequency of sexual intercourse in the previous 4 weeks (a single question in the McCoy test), we observed no differences between all groups at baseline.

At the end of the study, we observed a significant increase from baseline in the McCoy Questionnaire total score in women treated with DHEA and with HRT. Women receiving tibolone also increased their mean score, although this value, after 1 year of therapy, did not result in statistical significance in comparison with baseline. No differences were evaluated between the DHEA and HRT groups at the end of the entire follow-up (48.6 ±4.6 and 47.6 ± 3.66, respectively). Furthermore, we observed a significant increase in sexuality total score between the DHEA and vitamin D groups (48.6 ± 4.6 and 34.9 ±4.82, respectively, p 0.001), and the HRT and vitamin D groups (47.6 ± 3.66 and 34.9 ± 4.82, respectively, p 0.01) (Figure 1).

The relationship total score did not change from baseline to 1-year of treatment in any groups analyzed. The frequency of sexual intercourse in the last 4 weeks increased in women treated with DHEA, HRT and tibolone after 1-year therapy in comparison with the baseline score (p 0.01; p 0.05 and p 0.01, respectively). In addition, the extent of this modifi cation was the same for the DHEA, HRT and tibolone groups and no differences between these groups was observed at the end of treatment. The frequency of sexual intercourse did not change in women treated with vitamin D after 1 year and their scoring was significantly lower in comparison with those of women receiving hormone treatments (p 0.05) (Figure 1).

9.3 Discussion

The present study is evidence that symptomatic, early postmenopausal women receiving 1-year oral DHEA therapy at a daily dose of 10 mg improved their climacteric symptoms. The magnitude of this effect seems to be similar to the effect in women receiving 1-year therapy with tibolone or HRT. In addition, all hormone treatments used (DHEA, HRT and tibolone) improved the quality of sexual life in postmenopausal women, supporting the hypothesis that hormonal changes, during reproductive aging, negatively affect sexual function.

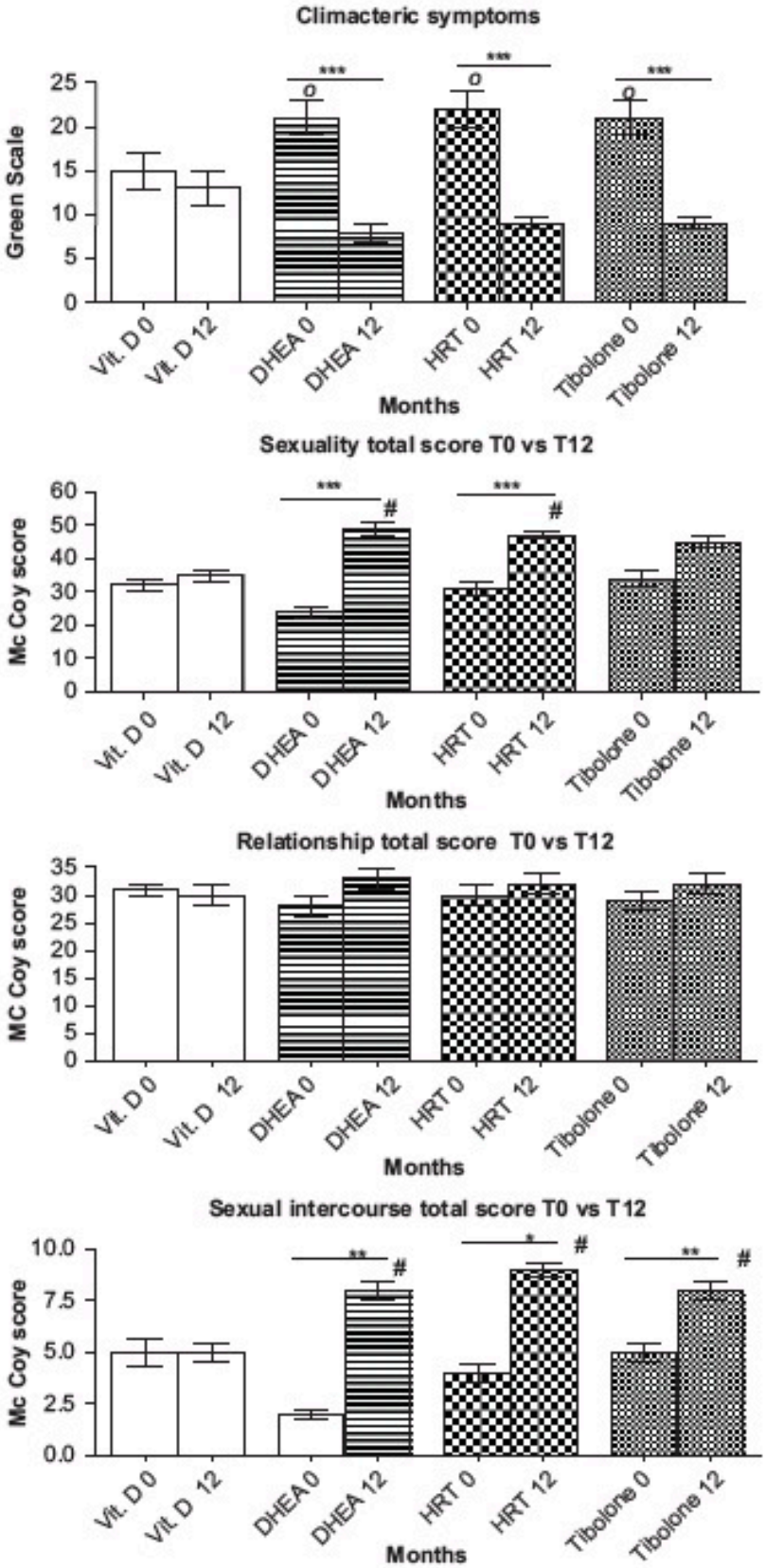
This finding has been achieved in healthy women that did not have criteria for the diagnosis of sexual dysfunction, but who opted for hormone treatment to reduce their climacteric symptoms.

Several trials have reported significant hormonal changes after DHEA administration in postmenopausal women. However, short follow-up and supraphysiological doses of DHEA (50 mg/day or higher) hampered these studies. Administration of a lower dose (25 mg/day) of DHEA produces positive effects on the hormonal milieu and on quality of life in early and late postmenopausal women, restoring estrogenic, progestogenic and androgenic tone in the range of fertile women (137).

In the present study, 1-year DHEA supplementation with 10 mg/day modified the endocrine milieu in postmenopausal women; this therapy enhanced plasma levels of estradiol and progesterone, it modified adrenal synthesis of cortisol (reduction) and allopregnanolone (increase) and it increased plasma concentration of β-endorphin, confirming our previous study and supporting also a direct action of DHEA on adrenal function.

The different impact of DHEA, in comparison with tibolone and HRT, on the androgenic circulating profile is also confirmed in the present study in which DHEA increased plasma Δ 5 and Δ 4 androgens levels, whereas estrogen – progestin therapy or tibolone did not induce any changes. The beneficial effects of DHEA on sexual function might be, at least in part, the direct consequence of this concomitant increase of estrogens, androgens and progesterone in symptomatic postmenopausal women. Indeed, sex steroids (estrogens, androgens and progesterone) positively affect critical aspects of sexual function in the central nervous system and, peripherally, in the genital tract. In addition, DHEA is a neurosteroid that acts directly as a modulator of neurotransmitter receptors, such as γ -aminobutyric acid type A, N -methyl- D -aspartate, and sigma-1 receptors.

Interestingly, although the effect on sexual function is similar for DHEA and HRT, using the McCoy Questionnaire, estrogen – progestin treatment induced higher (almost double) values of circulating estradiol than DHEA, supporting the hypothesis that estrogens are not the only mediators of sexual function in women. On the contrary, tibolone might increase sexual function through its unique receptor profile, as previously evidenced. To our knowledge, no previous studies have evaluated the effects of oral, low-dose DHEA replacement therapy on sexual function, and, in particular, have analyzed the frequency of sexual function. Only one study by Labrie and colleagues has reported that vaginal daily DHEA treatment, using the dose of 6.5 mg enhances arousal, desire and lubrication in healthy postmenopausal women (evaluated using the Abbreviated Sexual Function (ASFQ) questionnaires). In this regard, our findings are promising, although they need further exploration with a larger and more representative sample size.



Comparison of McCoy score (sexuality total score, relationship total score, sexual intercourse total score) between groups before the start of the treatment (T0) and at the end (T12). Climacteric symptoms were evaluated by the Greene Scale. Vit. D, vitamin D; DHEA, dehydroepiandrosterone; HRT, hormone replacement therapy. * , p 0.05; * * , p 0.01; * * * , p 0.001 vs. pretreatment values; #, p 0.05 DHEA or HRT vs. vitamin D

Table 2 Circulating levels of estradiol, progesterone, cortisol and sex hormone binding globulin (SHBG) at baseline and at 3, 6 and 12 months of therapy

Therapy group	*Baseline*	*3 months*	*6 months*	*12 months*
Estradiol (pg/ml)				
Placebo	19.29 ± 2.2	19.44 ± 2.2	18.52 ± 2.5	17.34 ± 2.1
DHEA	20.1 ± 3.2	21.47 ± 2.8**	24.91 ± 2.9**	27.48 ± 2.8**
HRT	18.07 ± 1.9	43.39 ± 6.8***	50.3 ± 7.8***	58.9 ± 9.0***
Tibolone	18.12 ± 2.1	20.18 ± 2.0	22.61 ± 2.6***	23.84 ± 2.3***
Progesterone (ng/ml)				
Placebo	0.40 ± 0.1	0.41 ± 0.1	0.39 ± 0.1	0.39 ± 0.08
DHEA	0.41 ± 0.1	0.42 ± 0.1	0.45 ± 0.1***	0.48 ± 0.1***
HRT	0.42 ± 0.07	0.44 ± 0.07	0.45 ± 0.07***	0.50 ± 0.07***
Tibolone	0.41 ± 0.09	0.41 ± 0.09	0.42 ± 0.08	0.42 ± 0.09
Cortisol (mg/l)				
Placebo	208 ± 15	211 ± 14	211 ± 14	212 ± 15
DHEA	204 ± 12	199 ± 12	193 ± 8**	188 ± 5***
HRT	214 ± 19	212 ± 20	211 ± 21	209 ± 21***
Tibolone	182 ± 15	189 ± 14*	178 ± 9***	175 ± 10***
SHBG (nmol/l)				
Placebo	54.72 ± 6.4	55.64 ± 4.2	57.16 ± 4.9	56.82 ± 4.64
DHEA	50.18 ± 11.00	52.26 ± 8.32	58.73 ± 9.00*	62.00 ± 7.5***
HRT	52.31 ± 15.9	57.11 ± 15.7*	62.3 ± 14.1*	63.54 ± 16.5**
Tibolone	55.51 ± 9.8	54.15 ± 8.7	53.68 ± 8.5*	51.05 ± 9.00***

DHEA, dehydroepiandrosterone; HRT, hormone replacement therapy
*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

Table 3 Circulating levels of dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), testosterone and free testosterone at baseline and at 3, 6 and 12 months of therapy

Therapy group	*Baseline*	*3 months*	*6 months*	*12 months*
DHEA (ng/ml)				
Placebo	1.92 ± 0.5	1.88 ± 0.5	1.83 ± 0.4	1.85 ± 0.5
DHEA	1.94 ± 0.5	2.04 ± 0.6	2.48 ± 0.5**	2.67 ± 0.6**
HRT	1.51 ± 0.6	1.51 ± 0.6	1.54 ± 0.6	1.54 ± 0.6
Tibolone	1.81 ± 0.7	1.83 ± 0.66	1.83 ± 0.7	1.84 ± 0.7
DHEAS (mg/ml)				
Placebo	0.71 ± 0.1	0.74 ± 0.1	0.72 ± 0.08	0.74 ± 0.1
DHEA	0.55 ± 0.1	0.65 ± 0.1	0.77 ± 0.1**	0.81 ± 0.2***
HRT	0.67 ± 0.1	0.67 ± 0.1	0.69 ± 0.08	0.73 ± 0.1
Tibolone	0.66 ± 0.1	0.71 ± 0.2	0.74 ± 0.2	0.7 ± 0.2
Testosterone (ng/ml)				
Placebo	0.36 ± 0.09	0.36 ± 0.07	0.38 ± 0.07	0.39 ± 0.07
DHEA	0.32 ± 0.1	0.39 ± 0.43	0.43 ± 0.1**	0.54 ± 0.06***
HRT	0.47 ± 0.09	0.35 ± 0.08**	0.37 ± 0.07**	0.38 ± 0.08**
Tibolone	0.41 ± 0.1	0.41 ± 0.1	0.38 ± 0.1	0.38 ± 0.1
Free testosterone (pg/ml)				
Placebo	1.47 ± 0.5	1.47 ± 0.6	1.49 ± 0.7	1.48 ± 0.5
DHEA	1.32 ± 0.2	1.33 ± 0.2	1.36 ± 0.2	1.32 ± 0.2
HRT	1.30 ± 0.3	1.28 ± 0.3	1.27 ± 0.3	1.27 ± 0.3
Tibolone	1.46 ± 0.5	1.47 ± 0.4	1.49 ± 0.4	1.49 ± 0.5

HRT, hormone replacement therapy
*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

Table 4 Circulating levels of androstenedione, 17-hydroxyprogesterone, allopregnanolone and β-endorphin at baseline and at 3, 6 and 12 months of therapy

Therapy group	*Baseline*	*3 months*	*6 months*	*12 months*
Androstenedione (ng/ml)				
Placebo	0.99 ± 1.1	0.93 ± 0.2	0.92 ± 0.1	0.91 ± 0.2
DHEA	0.93 ± 0.1	1.05 ± 0.1	1.21 ± 0.2**	1.28 ± 0.2***
HRT	0.98 ± 0.1	1.01 ± 0.2	1.04 ± 0.2	1.1 ± 0.2
Tibolone	1.01 ± 0.1	1.04 ± 0.1	1.08 ± 0.2	1.13 ± 0.2
17-hydroxyprogesterone (ng/ml)				
Placebo	0.52 ± 0.1	0.57 ± 0.2	0.52 ± 0.1	0.57 ± 0.1
DHEA	0.57 ± 0.06	0.57 ± 0.06	0.60 ± 0.06*	0.68 ± 0.05***
HRT	0.51 ± 0.1	0.64 ± 0.1*	0.66 ± 0.1**	0.80 ± 0.1***
Tibolone	0.59 ± 0.1	0.59 ± 0.1	0.63 ± 0.1	0.64 ± 0.1
Allopregnanolone (pg/ml)				
Placebo	224 ± 40	218 ± 44	229 ± 39	236 ± 39
DHEA	198 ± 18	199 ± 17	222 ± 18	223 ± 17
HRT	210 ± 23	240 ± 34**	254 ± 34***	270 ± 35***
Tibolone	197 ± 12	203 ± 15	210 ± 16**	227 ± 18***
β-endorphin (ng/ml)				
Placebo	18.33 ± 2.3	17.02 ± 2.5	17.48 ± 3.5	17.58 ± 3.7
DHEA	17.9 ± 2.9	22.39 ± 2.9*	28.33 ± 1.6***	35.27 ± 3.4***
HRT	19.5 ± 2.3	24.75 ± 3.1*	32.01 ± 2.6***	41.13 ± 4.8***
Tibolone	17.19 ± 4.1	20.2 ± 2.8	19.67 ± 3.0	23.89 ± 4.6***

DHEA, dehydroepiandrosterone; HRT, hormone replacement therapy

*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

10. CONCLUSION AND PROSPECTIVES

Over the past 10 years, hormone preparations of dehydroepiandrosterone (DHEA) have been available over the counter and have been sold as the 'fountain of youth'. This has raised concerns about the real clinical efficacy and the possible effects of such uncontrolled and widespread hormonal self-administration and the lack of quality control in this increasingly financially rewarding business.

Upcoming experimental and clinical studies renew the attention and the debate on one of the most attractive and controversial issues in the physiology of the aging process that is still far from being clearly defined by the scientific community.

The most relevant aspect meriting attention is certainly the controversial finding among the studies that investigate the correlation of the endogenous DHEA sulfate (DHEAS) level, the aging process or organ illness with the results coming from studies focusing on the effects of exogenous DHEAS administration on brain function, sexuality, cardiovascular health and metabolic syndrome.

Indeed, the marked age-related decline in serum DHEA and DHEAS has suggested that a deficiency of these steroids may be causally related to the development of a series of diseases that are generally associated with aging.

The postulated consequences of low DHEA levels include insulin resistance, obesity, cardiovascular disease, cancer, reduction of the immune defense system as well as psychosocial problems such as depression and a general deterioration in the sensation of well-being and cognitive function.

There is also growing evidence in the literature that a low DHEAS level, representing the most abundant sex steroids in plasma in humans (more than 1000 times higher than estradiol and testosterone levels), negatively correlates with the domains of sexual function in pre and postmenopausal women to a greater extent than testosterone levels. Nonetheless, a DHEAS cut-off level for defining androgen deficiency syndrome has not been established. Similarly, in a cross-sectional study, higher endogenous DHEAS levels are independently and favorably associated with several measures of cognitive function and well-being. As a consequence, DHEA replacement may seem an attractive treatment opportunity. Nevertheless, the analyses of clinical outcomes are far from being conclusive and many issues should still be addressed. Although DHEA preparations have been available in the market since the 1990s, there are very few definitive reports on the

biological functions of this steroid, and it is still the case that its regulation is unclear and its mechanisms of action largely yet to be established.

The great conundrum in this area is the lack of a clear mechanism of action of DHEA. It is known that this steroid serves as a precursor for estrogens and androgens, and many believe that DHEA is merely an inactive precursor pool for the formation of bioactive steroid hormones. To this extent, oral supplementation of DHEA in postmenopausal women results in the formation of significant amounts of 17b-estradiol and estrone, accompanied by increases in androstenedione, testosterone and dihydrotestosterone. This, plus the evidence that DHEA can also be converted into estrogens and other androgens within cells, supports the view that many actions of this steroid are indirect and mediated via estrogen and/or androgen receptors. However, the rate of DHEA metabolism into estrogen/testosterone in different tissues, the presence of enzyme regulators and the effect of the aging process on the intracrinology of DHEA require additional investigation. Indeed, DHEA administration in both early and late postmenopausal women directly affects the agerelated changes in adrenal enzymatic pathways and steroid synthesis, including DHEA and progesterone.

There is, also, increasing evidence for DHEA acting in its own right through a dedicated, although as yet unidentified, receptor. The existence of such a receptor for DHEA has been particularly investigated in brain tissue and in vascular cells.

Although there is still debate on DHEA receptors, these findings corroborate the evidence that DHEA is not just a pre-hormone of the adrenals, but rather a hormone in its own right, and that it modulates a series of biological processes, with a remarkable tropism for the central nervous system.

Clinically, the spectrum of women that would benefit from DHEA therapy is not clearly defined and nor is the dosage of hormone treatment. Whether DHEA therapy could be prescribed as a general anti-aging therapy or could be an alternative treatment for women suffering from androgen deficiency syndrome remains uncertain across studies. In particular, among symptomatic women, the spectrum of symptoms responding to DHEA requires further investigation, to define the type of sexual symptoms (e.g. decreased sexual function or hypoactive sexual desire disorder) and the degree of mood/cognitive symptoms that could be responsive to hormonal treatment.

Similarly, the definition of criteria for the choice of the starting dosage of DHEA to be prescribed in postmenopausal women needs further investigation: the extent of the symptoms, baseline DHEA(S) plasma levels, concomitant estrogen therapy or the combination of all the previous should be considered.

Plasma DHEA(S) levels at baseline and during treatment merit attention given that a cut-off value for DHEA(S) deficiency is not yet defined and the plasma level might not represent the rate of tissue conversion into estrogens or delta-4 androgens. This fact is also coupled with the route of administration of DHEA, given that oral, vaginal and parenteral administrations seem to induce different steroid concentrations in the plasma, with different clinical consequences and applications. All these findings may have far-reaching implications in the debate about the role of DHEA(S) in the female aging process and might reconcile discordant findings from basic science and clinical studies.

The lack of definitive evidence for biological mechanisms and the presence of only a few studies that address these emerging issues of DHEA therapy in postmenopausal women might encourage a new critical analysis of the available literature, evidencing current limits and incongruities. Concurrently, new clinical trials, specifically planned to relate to the biology of symptomatic postmenopausal women and designed for the translation of basic science into clinical practice, are now a required step to move forward the scientific debate on DHEA.

11. REFERENCES

1. Auchus RJ. Overview of dehydroepiandrosterone biosynthesis. Semin Reprod Med. 2004;22:281–288
2. Miller WL. Androgen biosynthesis from cholesterol to DHEA. Mol Cell Endocrinol. 2002;198:7–14
3. Rosa S, Duff C, Meyer M, Lang-Muritano M, Balercia G, Boscaro M, Topaloglu AK, Mioni R, Fallo F, Zuliani L, Mantero F, Schoenle EJ, Biason-Lauber A. P450c17 deficiency: clinical and molecular characterization of six patients. J Clin Endocrinol Metab. 2007;92:1000–1007.
4. Corpechot C, Robel P, Axelson M, Sjovall J, Baulieu EE. Characterization and measurement of dehydroepiandrosterone sulfate in rat brain. Proc Natl Acad Sci U S A. 1981;78:4704–4707.
5. Bair SR, Mellon SH. Deletion of the mouse P450c17 gene causes early embryonic lethality. Mol Cell Biol. 2004;24:5383–5390.
6. Migeon CJ, Keller AR, Lawrence B & Shepart TH II 1957 Dehydroepiandrosterone and androsterone levels in human plasma. Effect of age and sex: day-to-day and diurnal variations. Journal of Clinical Endocrinology and Metabolism 17 1051–1062.
7. Orentreich N, Brind JL, Rizer RL & Vogelman JH 1984 Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. Journal of Clinical Endocrinology and Metabolism 59 551–555.
8. F Labrie, V Luu-The, A Bélanger, S-X Lin, J Simard, G Pelletier and C Labrie Is dehydroepiandrosterone a hormone? J Endocrinol November 1, 2005 187 169-196
9. Labrie F, Bélanger A, Cusan L, Gomez JL & Candas B 1997c Marked decline in serum concentrations of adrenal C19 sex steroid precursors and conjugated androgen metabolites during aging. Journal of Clinical Endocrinology and Metabolism 82 2396–2402.
10. Bélanger A, Brochu M & Cliche J 1986 Levels of plasma steroid glucuronides in intact and castrated men with prostatic cancer. Journal of Clinical Endocrinology and Metabolism 62 812–815.
11. Bélanger A, Pelletier G, Labrie F, Barbier O & Chouinard S 2003 Inactivation of androgens by UDP-glucuronosyltransferase enzymes in humans. Trends in Endocrinology and Metabolism 14 473–479.
12. Turgeon D, Carrier JS, Lévesque E, Beatty BG, Bélanger A & Hum DW 2000 Isolation and characterization of the human UGT2B15 gene, localized within a cluster of UGT2B genes and pseudogenes on chromosome 4. Journal of Molecular Biology 295 489–504.
13. Carrier JS, Turgeon D, Journault K, Hum DW & Belanger A 2000 Isolation and characterization of the human UGT2B7 gene. Biochemical and Biophysical Research Communications 272 616–621.
14. B élanger B, Bélanger A, Labrie F, Dupont A, Cusan L & Monfette G 1989 Comparison of residual C-19 steroids in plasma and prostatic tissue of human, rat and guinea pig after castration: unique importance of extratesticular androgens in men. Journal of Steroid Biochemistry 32 695–698
15. Lacroix C, Fiet J, Benais JP, Gueux B, Bonete R, Villette JM, Gourmel B, Dreux C. Simultaneous radioimmunoassay of progesterone, androst-4-enedione, pregnenolone, dehydroepiandrosterone and 17-hydroxyprogesterone in specific regions of human brain. J Steroid Biochem. 1987;28:317–325.
16. Guazzo EP, Kirkpatrick PJ, Goodyer IM, Shiers HM, Herbert J.

Cortisol,dehydroepiandrosterone (DHEA), and DHEA sulfate in the cerebrospinal fluid of man: relation to blood levels and the effects of age. J Clin Endocrinol Metab. 1996;81:3951–3960.
17. Liere P, Pianos A, Eychenne B, Cambourg A, Liu S, Griffiths W, Schumacher M, Sjovall J, Baulieu EE. Novel lipoidal derivatives of pregnenolone and dehydroepiandrosterone and absence of their sulfated counterparts in rodent brain. J Lipid Res. 2004;45:2287–2302
18. Schumacher M, Liere P, Akwa Y, Rajkowski K, Griffiths W, Bodin K, Sjovall J, Baulieu EE. Pregnenolone sulfate in the brain: a controversial neurosteroid. Neurochem Int. 2008;52:522–540.
19. Corpechot C, Synguelakis M, Talha S, Axelson M, Sjovall J, Vihko R, Baulieu EE, Robel P. Pregnenolone and its sulfate ester in the rat brain. Brain Res. 1983;270:119–125
20. Weill-Engerer S, David JP, Sazdovitch V, Liere P, Eychenne B, Pianos A, Schumacher M, Delacourte A, Baulieu EE, Akwa Y. Neurosteroid quantification in human brain regions: comparison between Alzheimer's and nondemented patients. J Clin Endocrinol Metab. 2002;87:5138–5143.
21. Liere P, Pianos A, Eychenne B, Cambourg A, Liu S, Griffiths W, Schumacher M, Sjovall J, Baulieu EE. Novel lipoidal derivatives of pregnenolone and dehydroepiandrosterone and absence of their sulfated counterparts in rodent brain. J Lipid Res. 2004;45:2287–2302
22. Lanthier A, Patwardhan VV. Sex steroids and 5-en-3 beta-hydroxysteroids in specific regions of the human brain and cranial nerves. J Steroid Biochem. 1986;25:445–449
23. Weill-Engerer S, David JP, Sazdovitch V, Liere P, Eychenne B, Pianos A, Schumacher M, Delacourte A, Baulieu EE, Akwa Y. Neurosteroid quantification in human brain regions: comparison between Alzheimer's and nondemented patients. J Clin Endocrinol Metab. 2002;87:5138–5143
24. Fluck CE, Miller WL, Auchus RJ. Auchus, The 17, 20-lyase activity of cytochrome p450c17 from human fetal testis favors the delta5 steroidogenic pathway. J Clin Endocrinol Metab. 2003;88:3762–3766.
25. Conley AJ, Bird IM. The role of cytochrome P450 17 alpha-hydroxylase and 3 beta-hydroxysteroid dehydrogenase in the integration of gonadal and adrenal steroidogenesis via the delta 5 and delta 4 pathways of steroidogenesis in mammals. Biol Reprod. 1997;56:789–799.
26. Longcope C. Dehydroepiandrosterone metabolism. J Endocrinol. 1996;150 Suppl:S125–S127
27. Cutler GB, Jr, Glenn M, Bush M, Hodgen GD, Graham CE, Loriaux DL. Adrenarche: a survey of rodents, domestic animals, and primates. Endocrinology. 1978;103:2112–2118
28. van Weerden WM, Bierings HG, van Steenbrugge GJ, de Jong FH, Schroder FH. Adrenal glands of mouse and rat do not synthesize androgens. Life Sci. 1992;50:857–861
29. Baulieu EE. Neurosteroids: a novel function of the brain. Psychoneuroendocrinology. 1998;23:963–987
30. Baulieu EE, Robel P. Dehydroepiandrosterone and dehydroepiandrosterone sulfate as neuroactive neurosteroids. J Endocrinol. 1996;150 Suppl:S221–S239
31. Compagnone NA, Bulfone A, Rubenstein JL, Mellon SH. Steroidogenic enzyme P450c17 is expressed in the embryonic central nervous system. Endocrinology. 1995;136:5212–5223
32. Hojo Y, Hattori TA, Enami T, Furukawa A, Suzuki K, Ishii HT, Mukai H, Morrison JH, Janssen WG, Kominami S, Harada N, Kimoto T, Kawato S. Adult male rat

hippocampus synthesizes estradiol from pregnenolone by cytochromes P45017alpha and P450 aromatase localized in neurons. Proc Natl Acad Sci U S A. 2004;101:865–870
33. Kimoto T, Tsurugizawa T, Ohta Y, Makino J, Tamura H, Hojo Y, Takata N, Kawato S. Neurosteroid synthesis by cytochrome p450-containing systems localized in the rat brain hippocampal neurons: N-methyl-D-aspartate and calcium-dependent synthesis. Endocrinology. 2001;142:3578–3589.
34. Do Rego JL, Tremblay Y, Luu-The V, Repetto E, Castel H, Vallarino M, Belanger A, Pelletier G, Vaudry H. Immunohistochemical localization and biological activity of the steroidogenic enzyme cytochrome P450 17alpha-hydroxylase/C17, 20-lyase (P450C17) in the frog brain and pituitary. J Neurochem. 2007;100:251–268
35. Cibaly C, Patte-Mensah C, Mensah-Nyagan AG. Molecular and neurochemical evidence for the biosynthesis of dehydroepiandrosterone in the adult rat spinal cord. J Neurochem. 2005;93:1220–1230
36. Kishimoto Y, Hoshi M. Dehydroepiandrosterone sulphate in rat brain: incorporation from blood and metabolism in vivo. J Neurochem. 1972;19:2207–2215.
37. Schumacher M, Liere P, Akwa Y, Rajkowski K, Griffiths W, Bodin K, Sjovall J, Baulieu EE. Pregnenolone sulfate in the brain: a controversial neurosteroid. Neurochem Int. 2008;52:522–540.
38. Webb SJ, Geoghegan TE, Prough RA, Michael Miller KK. The biological actions of dehydroepiandrosterone involves multiple receptors. Drug Metab Rev. 2006;38:89–116.
39. Weill-Engerer S, David JP, Sazdovitch V, Liere P, Eychenne B, Pianos A, Schumacher M, Delacourte A, Baulieu EE, Akwa Y. Neurosteroid quantification in human brain regions: comparison between Alzheimer's and nondemented patients. J Clin Endocrinol Metab. 2002;87:5138–5143.
40. Widstrom RL, Dillon JS. Is there a receptor for dehydroepiandrosterone or dehydroepiandrosterone sulfate? Semin Reprod Med. 2004;22:289–298
41. Labrie F. Adrenal androgens and intracrinology. Semin Reprod Med. 2004;22:299–309
42. Chalbot S, Morfin R. Dehydroepiandrosterone metabolites and their interactions in humans. Drug Metabol Drug Interact. 2006;22:1–23
43. Baulieu EE. Neurosteroids: of the nervous system, by the nervous system, for the nervous system. Recent Prog Horm Res. 1997;52:1–32
44. Baulieu EE. Neurosteroids: a novel function of the brain. Psychoneuroendocrinology. 1998;23:963–987
45. Bergeron R, de Montigny C, Debonnel G. Potentiation of neuronal NMDA response induced by dehydroepiandrosterone and its suppression by progesterone: effects mediated via sigma receptors. J Neurosci. 1996;16:1193–1202
46. Compagnone NA, Mellon SH. Neurosteroids: biosynthesis and function of these novel neuromodulators. Front Neuroendocrinol. 2000;21:1–56.
47. Maurice T, Gregoire C, Espallergues J. Neuro(active)steroids actions at the neuromodulatory sigma1 (sigma1) receptor: biochemical and physiological evidences, consequences in neuroprotection. Pharmacol Biochem Behav. 2006;84:581–597.
48. Mellon SH, Griffin LD, Compagnone NA. Biosynthesis and action of neurosteroids. Brain Res Brain Res Rev. 2001;37:3–12.
49. Rupprecht R. Neuroactive steroids: mechanisms of action and neuropsychopharmacological properties. Psychoneuroendocrinology. 2003;28:139–168
50. Rupprecht R, Michele Fdi, Hermann B, Strohle A, Lancel M, Romeo E, Holsboer F. Neuroactive steroids: molecular mechanisms of action and implications for neuropsychopharmacology. Brain Res Brain Res Rev. 2001;37:59–67
51. Rupprecht R, Holsboer F. Neuroactive steroids: mechanisms of action and

neuropsychopharmacological perspectives. Trends Neurosci. 1999;22:410
52. Imamura M, Prasad C. Modulation of GABA-gated chloride ion influx in the brain by dehydroepiandrosterone and its metabolites. Biochem Biophys Res Commun. 1998;243:771–775.
53. Majewska MD. Neurosteroids: endogenous bimodal modulators of the GABAA receptor. Mechanism of action and physiological significance. Prog Neurobiol. 1992;38:379–395
54. Gonzalez-Alvear GM, Werling LL. Regulation of (3H)dopamine release from rat striatal slices by sigma receptor ligands. J Pharmacol Exp Ther. 1994;271:212–219.
55. Bergeron R, de Montigny C, Debonnel G. Potentiation of neuronal NMDA response induced by dehydroepiandrosterone and its suppression by progesterone: effects mediated via sigma receptors. J Neurosci. 1996;16:1193–1202.
56. Glei DA, Goldman N, Weinstein M, Liu IW. Dehydroepiandrosterone sulfate (DHEAS) and health: does the relationship differ by sex? Exp Gerontol. 2004;39:321–3
57. George O, Vallee M, Le Moal M, Mayo W. Neurosteroids and cholinergic systems: implications for sleep and cognitive processes and potential role of age-related changes. Psychopharmacology (Berl) 2006;186:402–413.
58. Rhodes ME, Li PK, Flood JF, Johnson DA. Enhancement of hippocampal acetylcholine release by the neurosteroid dehydroepiandrosterone sulfate: an in vivo microdialysis study. Brain Res. 1996;733:284–286
59. Urani A, Privat A, Maurice T. The modulation by neurosteroids of the scopolamine-induced learning impairment in mice involves an interaction with sigma1 (sigma1) receptors. Brain Res. 1998;799:64–77
60. Rhodes ME, Li PK, Burke AM, Johnson DA. Enhanced plasma DHEAS, brain acetylcholine and memory mediated by steroid sulfatase inhibition. Brain Res. 1997;773:28–32.
61. Laurine E, Lafitte D, Gregoire C, Seree E, Loret E, Douillard S, Michel B, Briand C, Verdier JM. Specific binding of dehydroepiandrosterone to the N terminus of the microtubule-associated protein MAP2. J Biol Chem. 1991;278:29979–29986.
62. Liu D, Dillon JS. Dehydroepiandrosterone activates endothelial cell nitric-oxide synthase by a specific plasma membrane receptor coupled to Galpha(i2,3) J Biol Chem. 2002;277:21379–21388.
63. Kohalmy K, Tamasi V, Kobori L, Sarvary E, Pascussi JM, Porrogi P, Rozman D, Prough RA, Meyer UA, Monostory K. Dehydroepiandrosterone induces human CYP2B6 through the constitutive androstane receptor. Drug Metab Dispos. 2007;35:1495–1501
64. Tamasi V, Miller KK, Ripp SL, Vila E, Geoghagen TE, Prough RA. Modulation of receptor phosphorylation contributes to activation of peroxisome proliferator activated receptor alpha by dehydroepiandrosterone and other peroxisome proliferators. Mol Pharmacol. 2008;73:968–976.
65. Webb SJ, Geoghegan TE, Prough RA, Michael Miller KK. The biological actions of dehydroepiandrosterone involves multiple receptors. Drug Metab Rev. 2006;38:8
66. Jellinck PH, Kaufmann M, Gottfried-Blackmore A, McEwen BS, Jones G, Bulloch K. Selective conversion by microglia of dehydroepiandrosterone to 5-androstenediol-A steroid with inherent estrogenic properties. J Steroid Biochem Mol Biol. 2007 Nov-Dec;107(3-5):156-62
67. Joëls M. Steroid hormones and excitability in the mammalian brain. Front Neuroendocrinol. 1997 Jan;18(1):2-48.
68. Mukai H, Tsurugizawa T, Ogiue-Ikeda M, Murakami G, Hojo Y, Ishii H, Kimoto T, Kawato S. Local neurosteroid production in the hippocampus: influence on synaptic plasticity of memory. Neuroendocrinology. 2006;84(4):255-63

69. Wolf OT, Kirschbaum C. Actions of dehydroepiandrosterone and its sulfate in the central nervous system: effects on cognition and emotion in animals and humans. Brain Res Brain Res Rev. 1999 Nov;30(3):264-88.
70. Perez-Neri I, Montes S, Ojeda-Lopez C, Ramirez-Bermudez J, Rios C. Modulation of neurotransmitter systems by dehydroepiandrosterone and dehydroepiandrosterone sulfate: Mechanism of action and relevance to psychiatric disorders. Prog Neuropsychopharmacol Biol Psychiatry. 2008
71. Svec F, Hilton CW, Wright B, Browne E, Porter JR. The effect of DHEA given chronically to Zucker rats. Proc Soc Exp Biol Med. 1995;209:92–97
72. Svec F, Porter J. The effect of dehydroepiandrosterone (DHEA) on Zucker rat food selection and hypothalamic neurotransmitters. Psychoneuroendocrinology. 1997;22 Suppl 1:S57–S62.
73. Murray HE, Gillies GE. Differential effects of neuroactive steroids on somatostatin and dopamine secretion from primary hypothalamic cell cultures. J Neuroendocrinol. 1997;9:287–295.
74. Sicard F, Krug AW, Ziegler CG, Sperber S, Ehrhart-Bornstein M, Bornstein SR. Role of DHEA and growth factors in chromaffin cell proliferation. Ann N Y Acad Sci. 2006;1073:312–316
75. Sicard F, Ehrhart-Bornstein M, Corbeil D, Sperber S, Krug AW, Ziegler CG, Rettori V, McCann SM, Bornstein SR. Bornstein, Age-dependent regulation of chromaffin cell proliferation by growth factors, dehydroepiandrosterone (DHEA), and DHEA sulfate. Proc Natl Acad Sci U S A. 2007;104:2007–2012.
76. Ziegler CG, Sicard F, Sperber S, Ehrhart-Bornstein M, Bornstein SR, Krug AW. DHEA reduces NGF-mediated cell survival in serum-deprived PC12 cells. Ann N Y Acad Sci. 2006;1073:306–311.
77. Ziegler CG, Sicard F, Lattke P, Bornstein SR, Ehrhart-Bornstein M, Krug AW. Dehydroepiandrosterone induces a neuroendocrine phenotype in nerve growth factor-stimulated chromaffin pheochromocytoma PC12 cells. Endocrinology. 2008;149:320–328
78. Charalampopoulos I, Dermitzaki E, Vardouli L, Tsatsanis C, Stournaras C, Margioris AN, Gravanis A. Dehydroepiandrosterone sulfate and allopregnanolone directly stimulate catecholamine production via induction of tyrosine hydroxylase and secretion by affecting actin polymerization. Endocrinology. 2005;146:3309–3318
79. Majewska M.D., Neurosteroids: endogenous bimodal modulators of the GABA A receptors: Mechanism of action and physiological significance, Prog Neurobiol, Volume: 38, (1992), pp. 379--395
80. Genazzani A.R., Petraglia F., Bernardi F., Casarosa E., Salvestroni C., Tonetti A., et al. Circulating levels of allopregnanolone in humans: gender, age and endocrine influences, J Clin Endocrinol Metab, Volume: 83, (1998), pp. 2099--2103
81. Genazzani A.R., Bernardi F., Stomati M., Monteleone P., Luisi S., Rubino S., et al. Effects of estradiol and raloxifene analog on brain, adrenal and serum allopregnanolone content in fertile and ovariectomized female rats, Neuroendocrinol, Volume: 72, (2000), pp. 162--170
82. Stomati M., Bernardi F., Luisi S., Puccetti S., Casarosa E., Liut M., et al. Conjugated equine estrogens, estrone sulphate and estradiol valerate oral administration in ovariectomized rats: effects on central and peripheral allopregnanolone and beta-endorphin, Maturitas, Volume: 43, (2002), pp. 195--206
83. Bernardi F, Casarosa E, Pluchino N, Palumbo M, Genazzani AD, Luisi S, Genazzani AR Effect of dehydroepiandrosterone on central and peripheral levels of allopregnanolone and beta-endorphin. Fertil Steril. 2005 Apr;83 Suppl 1:1161-8.
84. Brandling-Bennett, E.M., Blasberg, M.E., Clark, A.S. Paced mating behavior in female

rats in response to different hormone priming regimens. Horm. Behav. 1999; 35:144-154.
85. Frye CA. Neurosteroids' effects and mechanisms for social, cognitive, emotional, and physical functions. Psychoneuroendocrinology. 2009 Dec
86. Clayton AH. Sexual function and dysfunction in women. Psychiatr Clin North Am. 2003 ;26(3):673-82.
87. Beach, F.A., Sexual attractivity, proceptivity, and receptivity in female mammals. Horm. Behav. 1976; 7,105-138.
88. Madlafousek, J., Hlinak Z., Importance of female's precopulatory behaviour for the primary initiation of male's copulatory behaviour in the laboratory rat. Behaviour 1983; 86, 237-249.
89. Pfaus JG, Kippin TE, Coria-Avila G. What can animal models tell us about human sexual response? Annu Rev Sex Res 2003; 14, 1-63
90. Meyerson, B.J., Lindström, L.H. Sexual motivation in the female rat. A methodological study applied to the investigation of the effect of estradiol benzoate. Acta Physiol. Scand. 1973; 389, 1-80.
91. Vega Matuszczyk, J., Larsson, K.,. Role of androgen, estrogen and sexual experience on the female rat's partner preference. Physiol. Behav. 1991; 50, 139-142.
92. Zipse LR, Brandling-Bennet EM, Clark AS. Paced mating behaviour in the naturally cicling and the hormone-treated female rat. Physiol Behav 2000; 70:205-9.
93. Hardy, D.F., DeBold, J.F.,. The relationship between levels of exogenous hormones and the display of lordosis by the female rat. Horm. Behav. 1971; 2, 287-297.
94. Ågmo, A., Turi, A.L., Ellingsen, E., Kaspersen, H., 2004. Preclinical models of sexual desire: conceptual and behavioral analyses. Pharmacol. Biochem. Behav. 78, 379-404
95. Clark, A.S., Guarraci, F.A., Megroz, A.B., Porter, D.M., Henderson L.P.,. The display of sexual behaviors by female rats administered ICI 182,780. Horm. Behav. 2003; 43, 454-464
96. Herbert J 1995 Neuropeptides, stress and sexuality: towards a new psychopharmacology. In The Pharmacology of Sexual Function and Dysfunction, pp77–92. Ed. J Bancroft. Amsterdam: Excerpta Medica International Congress Series 1075.
97. Argiolas A 1999 Neuropeptide and sexual behaviour. Neuroscience and Biobehavioral Reviews 23 1127–1142.
98. Martel C, Rhéaume E, Takahashi M, Trudel C, Couët J, Luu-The V, Simard J, Labrie F Distribution of 17 beta-hydroxysteroid dehydrogenase gene expression and activity in rat and human tissues. J Steroid Biochem Mol Biol. 1992;41:597-603.
99. Maninger N, Wolkowitz OM, Reus VI, Epel ES, Mellon SH. Neurobiological and neuropsychiatric effects of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). Front Neuroendocrinol. 2009;30:65-9
100. Mizuno T, Yotsuyanagi S, Kondo Y, Komatsu K, Ishiura Y, Nakamura Y, Nagasaka Y, Yokoyama O, Namiki M. Dehydroepiandrosterone alleviates copulatory disorder induced by social stress in male rats. J Sex Med. 2006;3:612-8
101. Erskine, M.S.,. Solicitation behavior in the estrous female rat: a review. Horm. Behav., 1989, 23, 473-502
102. Herbert J 1995 Neuropeptides, stress and sexuality: towards a new psychopharmacology. In The Pharmacology of Sexual Function and Dysfunction, pp77–92. Ed. J Bancroft. Amsterdam: Excerpta Medica International Congress Series 1075.
103. Argiolas A 1999 Neuropeptide and sexual behaviour. Neuroscience and Biobehavioral Reviews 23 1127–1142.
104. Martel C, Rhéaume E, Takahashi M, Trudel C, Couët J, Luu-The V, Simard J, Labrie F

Distribution of 17 beta-hydroxysteroid dehydrogenase gene expression and activity in rat and human tissues. J Steroid Biochem Mol Biol. 1992;41:597-603.
105. Maninger N, Wolkowitz OM, Reus VI, Epel ES, Mellon SH. Neurobiological and neuropsychiatric effects of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). Front Neuroendocrinol. 2009;30:65-9
106. Fava M, Rosenbaum JF, MacLaughlin RA, Tesar GE, Pollack MH, Cohen LS, Hirsch M. Dehydroepiandrosterone-sulfate/cortisol ratio in panic disorder. Psychiatry Res. 1989;28:345–350
107. Leblhuber F, Windhager E, Neubauer C, Weber J, Reisecker F, Dienstl E. Antiglucocorticoid effects of DHEA-S in Alzheimer's disease. Am J Psychiatry. 1992;149:1125–1126.
108. Leblhuber F, Neubauer C, Peichl M, Reisecker F, Steinparz FX, Windhager E, Dienstl E. Age and sex differences of dehydroepiandrosterone sulfate (DHEAS) and cortisol (CRT) plasma levels in normal controls and Alzheimer's disease (AD) Psychopharmacology (Berl) 1993;111:23–26
109. Wolkowitz OM, Reus VI, Manfredi F, Roberts E. Antiglucocorticoid effects of DHEA-S in Alzheimer's Disease (Reply) Am J Psychiatry. 1992;149:1126.
110. Wolkowitz OM, Epel ES, Reus VI. Stress hormone-related psychopathology: pathophysiological and treatment implications. World J Biol Psychiatry. 2001;2:115–143
111. Lupien S, Sharma S, Arcand JF, Schwartz G, Nair NPV, Meaney MJ, Hauger RL. Dehydroepiandrosterone-sulfate (DHEA-S) levels, cortisol levels and cognitive function in elderly human subjects. International Society of Psychoneuroendocrinology, 1995:24
112. Carvalhaes-Neto N, Huayllas MK, Ramos LR, Cendoroglo MS, Kater CE. Cortisol,DHEAS and aging: resistance to cortisol suppression in frail institutionalized elderly. J Endocrinol Invest. 2003;26:17–22.
113. McEwen BS. Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. Ann N Y Acad Sci. 2004;1032:1–7
114. Seeman TE, McEwen BS, Rowe JW, Singer BH. Allostatic load as a marker of cumulative biological risk: MacArthur studies of successful aging. Proc Natl Acad Sci U S A. 2001;98:4770–4775.
115. Barrett-Connor E, von Muhlen D, Laughlin GA, Kripke A. Endogenous levels of dehydroepiandrosterone sulfate, but not other sex hormones, are associated with depressed mood in older women: the Rancho Bernardo Study. J Am Geriatr Soc. 1999;47:685–691
116. Michael A, Jenaway A, Paykel ES, Herbert J. Altered salivary dehydroepiandrosterone levels in major depression in adults. Biol Psychiatry. 2000;48:989–995.
117. Olff M, de Vries GJ, Guzelcan Y, Assies J, Gersons BP. Changes in cortisol and DHEA plasma levels after psychotherapy for PTSD. Psychoneuroendocrinology. 2007;32:619–626.
118. Rasmusson AM, Vasek J, Lipschitz DS, Vojvoda D, Mustone ME, Shi Q, Gudmundsen G, Morgan CA, Wolfe J, Charney DS. An increased capacity for adrenal DHEA release is associated with decreased avoidance and negative mood symptoms in women with PTSD. Neuropsychopharmacology. 2004;29:1546–1557.
119. Yehuda R, Brand SR, Golier JA, Yang RK. Clinical correlates of DHEA associated with post-traumatic stress disorder. Acta Psychiatr Scand. 2006;114:187–193
120. Wolkowitz OM, Reus VI, Roberts E, Manfredi F, Chan T, Raum WJ, Ormiston S, Johnson R, Canick J, Brizendine L, Weingartner H. Dehydroepiandrosterone (DHEA) treatment of depression. Biol Psychiatry. 1997;41:311–318

121. Wolkowitz OM, Reus VI, Keebler A, Nelson N, Friedland M, Brizendine L, Roberts E. Double-blind treatment of major depression with dehydroepiandrosterone (DHEA) Am J Psychiatry. 1999;156:646–649
122. Davis SR, Davison SL, Donath S, Bell RJ 2005 Circulating androgen levels and self-reported sexual function in women. JAMA 294: 91–96
123. Basson R, Brotto LA, Petkau AJ, Labrie F 2010 Role of androgens in women's sexual dysfunction. Menopause 17:962–971
124. Davis SR, Shah SM, McKenzie DP, Kulkarni J, Davison SL, Bell RJ 2008 Dehydroepiandrosterone sulfate levels are associated with more favorable cognitive function in women. J Clin Endocrinol Metab 93:801–808
125. Panjari M, Bell RJ, Jane F, Wolfe R, Adams J, Morrow C, Davis SR 2009 A randomized trial of oral DHEA treatment for sexual function, well-being, and menopausal symptoms in postmenopausal women with low libido. J Sex Med 6:2579–2590
126. Wolf OT, Neumann O, Hellhammer DH, Geiben AC, Strasburger CJ, Dressendo¨ rfer RA, Pirke KM, Kirschbaum C 1997 Effects of a two-week physiological dehydroepiandrosterone substitution on cognitive performance and well-being in healthy elderly women and men. J Clin Endocrinol Metab 82:2363–2367
127. Baulieu EE, Thomas G, Legrain S, Lahlou N, Roger M, Debuire B, Faucounau V, Girard L, HervyMP, Latour F, LeaudMC, Mokrane A, Pitti-Ferrandi H, Trivalle C, de Lacharrie`re O, Nouveau S, Rakoto-Arison B, Souberbielle JC, Raison J, Le Bouc Y, Raynaud A, Girerd X, Forette F 2000 Dehydroepiandrosterone (DHEA), DHEA sulfate, and aging: contribution of the DHEAge Study to a sociobiomedical issue. Proc Natl Acad Sci USA 97:4279–4284
128. Hackbert L, Heiman JR 2002 Acute dehydroepiandrosterone (DHEA) effects on sexual arousal in postmenopausal women. J Womens Health Gend Based Med 11:155–162
129. Kritz-Silverstein D, von Mu¨ hlen D, Laughlin GA, Bettencourt R 2008 Effects of dehydroepiandrosterone supplementation on cognitive function and quality of life: the DHEA and Well-Ness (DAWN) Trial. J Am Geriatr Soc 56:1292–1298
130. Labrie F, Archer D, Bouchard C, Fortier M, Cusan L, Gomez JL, Girard G, Baron M, Ayotte N, Moreau M, Dube´ R,Coˆ te´ I, Labrie C, Lavoie L, Berger L, Gilbert L, Martel C, Balser J 2009 Effect of intravaginal dehydroepiandrosterone (Prasterone) on libido and sexual dysfunction in postmenopausal women. Menopause 16: 923–931
131. Nair KS, Rizza RA, O'Brien P, Dhatariya K, Short KR, Nehra A, Vittone JL, Klee GG, Basu A, Basu R, Cobelli C, Toffolo G, Dalla ManC, Tindall DJ, Melton 3rd LJ, Smith GE, Khosla S, JensenMD 2006DHEAin elderly women and DHEA or testosterone in elderly men. N Engl J Med 355:1647–1659
132. Morales AJ, Nolan JJ, Nelson JC, Yen SS 1994 Effects of replacement dose of dehydroepiandrosterone in men and women of advancing age. J Clin Endocrinol Metab (Erratum (1995) 80:2799) 78:1360–1367
133. Mortola JF, Yen SS 1990 The effects of oral dehydroepiandrosterone on endocrine-metabolic parameters in postmenopausal women. J Clin Endocrinol Metab 71:696–704
134. Bloch M, Schmidt PJ, Danaceau MA, Adams LF, Rubinow DR 1999 dehydroepiandrosterone treatment of midlife dysthymia. Biol Psychiatry 45:1533–1541
135. McCoy NL. McCoy Female Sexuality Questionnaire. Qual Life Res 2000;9:739 – 45
136. Rellini AH, Nappi RE, Vaccaro P, e t al. Validation of the McCoy female Sexuality Questionnaire in an Italian sample. Arch Sex Behav 2005;34:641 – 7
137. Pluchino N, Ninni F, Stomati M, et al . One year therapy with 10 mg/die DHEA alone or in combination with HRT in postmenopausal women: effects on hormonal milieu. Maturitas 2008;59:296 – 303